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- (71) Applicant (*for all designated States except US*): **PRO-CHON BIOTECH LTD.** [IL/IL]; P.O. Box 1482, 76114 Rehovot (IL).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **EREN, Doron** [IL/IL]; Moshav Netaim, 46870 (IL). **ZALIANI, Andrea** [DE/DE]; Dorotheenstrasse 23, 22301 Hamburg (DE). **PE'ER, David** [IL/IL]; Hanasi Harishon Street 34, Rehovot 76303 (IL). **BOGIN, Oren** [IL/IL]; Moshav Ganei Yochanan 160, 76922 (IL). **YAYON, Avner** [IL/IL]; Moshav Sitria # 104, 76834 (IL).
- (74) Agent: **WEBB, Cynthia**; Webb & Associates, P.O. Box 2189, 76121 Rehovot (IL).
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(54) Title: PROTEIN TYROSINE KINASE INHIBITORS

(57) Abstract: The present invention provides heterocyclic compounds which can act as protein tyrosine kinase inhibitors. Preferred embodiments include both novel and known substituted 2-aryl benzimidazole and benzofuro [3,2-c] quinoline compounds. Methods of utilizing preferred compounds in preparation of medicaments for the treatment of proliferative, skeletal and metabolic diseases and disorders associated with abnormal protein tyrosine kinase activity, including cancer and skeletal dysplasia, are provided.



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PROTEIN TYROSINE KINASE INHIBITORS

FIELD OF THE INVENTION

The present invention relates to inhibition of abnormal protein tyrosine kinase (PTK)

5 activity, particularly to the use of certain heterocyclic compounds as protein tyrosine kinase inhibitors, and to some novel heterocyclic compounds.

BACKGROUND OF THE INVENTION

A wide variety of biological processes involve complex cellular communication mechanisms. One of the primary means of continual exchange of information between

10 cells and their internal and external environments is via the secretion and specific binding of peptide growth factors. Growth factors exert pleiotropic effects and play important roles in the development of multicellular organisms regulating cell growth, differentiation and migration as well in oncogenesis. Many of these factors mediate their effects by binding to specific cell surface receptors that trigger an enzymatic signal transduction cascade from
15 the cell membrane to the cell nucleus, resulting in specific gene regulation, leading to diverse cellular responses.

Protein kinases

One of the key biochemical mechanisms of signal transduction involves the reversible phosphorylation of intracellular protein substrates, which enables regulation of the activity

20 of mature proteins by altering their structure and function.

Protein kinases ("PKs") are enzymes that catalyze the phosphorylation of hydroxyl groups on tyrosine, serine and threonine residues of proteins. Abnormal PK activity has been related to a host of disorders, ranging from relatively non-life threatening diseases such as psoriasis to extremely virulent diseases such as glioblastoma. These kinases largely fall

25 into two groups: serine/threonine kinases, which are specific for phosphorylation of serine and threonine residues, and tyrosine kinases (PTK), specific for phosphorylation of tyrosine residues. Some kinases, referred to as "dual specificity" kinases, are able to phosphorylate tyrosine and serine/threonine residues.

Protein kinases are also characterized by their location within the cell. Some kinases are

30 transmembrane receptor proteins capable of binding ligand(s) external to the cell membrane. Ligand binding alters the receptor protein kinase's catalytic activity. Others

are non-receptor proteins lacking a transmembrane domain. Non-receptor protein kinases can be found in a variety of cellular compartments from the inner-surface of the cell membrane to the nucleus. Still others are ectokinases that have their catalytic domain on the extracellular (ecto) portion of the transmembrane protein or which are secreted as
5 soluble extracellular proteins.

Many kinases are involved in regulatory cascades in which their substrates may include other kinases whose activities are regulated by their phosphorylation state. Ultimately the activity of a downstream effector is modulated by its phosphorylation resulting from the activation of such a pathway. Kinases regulate all aspects of cellular function including cell
10 growth, migration, differentiation, apoptosis, gene expression, muscle contraction, glucose metabolism, cellular protein synthesis, and regulation of the cell cycle.

Receptor protein tyrosine kinases (RPTKs) are a subclass of transmembrane-spanning receptors endowed with intrinsic, ligand-stimulatable tyrosine kinase activity. When mutated or altered structurally, RPTKs can become potent oncoproteins, causing cellular
15 transformation. Conversely, RPTK activity in resting, untransformed cells is normally tightly controlled. In principle, oncogenic deregulation of all RPTKs involved in cancer, results from a relief or a perturbation of one or several of the auto-control mechanisms that ensure the normal repression of the catalytic domains. A little more than half of the known RPTKs have been repeatedly found in either mutated or overexpressed forms associated
20 with human malignancies (including sporadic cases) as listed in Table 1 of Blume-Jensen and Hunter (2001). RPTK overexpression leads to constitutive kinase activation by increasing the concentration of dimers. Important examples are the Neu/ErbB2 and epidermal growth factor receptor (EGFR), which are often amplified in breast (reviewed in Stern, 2000) and lung carcinomas and FGFR3 implicated in multiple myeloma, cervical
25 and bladder cancer.

Fibroblast Growth Factors

Fibroblast growth factors (FGFs) constitute a family of over twenty structurally related polypeptides that are developmentally regulated and expressed in a wide variety of tissues. FGFs stimulate proliferation, migration and differentiation of cells of mesenchymal and
30 neuroectodermal origin and play a major role in skeletal and limb development, wound healing, hematopoiesis, angiogenesis, and tissue repair as well as in tumorigenesis.

- The biological action of FGFs is mediated by specific cell surface receptors (FGFRs) that possess intrinsic tyrosine kinase activity and are phosphorylated upon binding of FGF. These proteins consist of an extracellular ligand binding domain, a single transmembrane domain and an intracellular tyrosine kinase domain. The FGFR extracellular region
- 5 contains three immunoglobulin-like (Ig-like) loops or domains (D1, D2 and D3), an acidic box, and a heparin-binding domain. Four FGFR genes that encode for multiple receptor protein variants are known. A new FGFR, designated FGFR5, lacking an intracellular domain has recently been identified (Sleeman *et al.*, 2001). The multiple alternatively spliced variants of FGFRs are designated as α -, β -, or γ -type.
- 10 Heparan sulfate proteoglycans (HSPGs) are required for high affinity interaction and activation of all members of the FGF family. Tissue-specific expression of heparan sulfate structural variants confers ligand-receptor specificity and activity of FGFs.

FGF-related Disease

- FGFs and their receptors (FGFRs) play a key role during growth, development and repair
- 15 of the human skeleton and are also implicated in chemotaxis, angiogenesis, apoptosis, and spatial patterning (Burgess and Maciag, 1989; Martin *et al.*, 1998; Martin, 1998). A growing number of skeletal abnormalities have been shown to result from mutations in FGFRs. Specific point mutations in different domains of FGFR3 are associated with autosomal dominant human skeletal disorders, such as achondroplasia - the most common
- 20 form of human dwarfism, hypochondroplasia, severe achondroplasia with developmental delay, acanthosis nigricans (SADDAN), and thanatophoric dysplasia. FGFR3 mutations have also been described in Muenke coronal craniosynostosis and Crouzon syndrome with acanthosis nigricans (reviewed in Webster and Donoghue, 1997 and Vajo, *et al.*, 2000). Certain mutations in FGFRs result in overactivation, i.e., constitutive activation, of the
- 25 mutated receptors and increased RPTK activity, rendering cells and tissue unable to differentiate. Specifically, the achondroplasia mutations result in enhanced stabilization of the mutated receptor, dissociating receptor activation from down-regulation, leading to restrained chondrocyte maturation and aberrant bone formation (Monson-Orran, *et al.* 2000).
- 30 There is accumulating evidence for FGFR activating mutations in various types of cancer. Constitutively activated FGFR3 in a large proportion of two common epithelial cancers, bladder and cervix, as well as in multiple myeloma, provide evidence of an oncogenic role

for FGFR3 (Chesi, *et al.*, 1997; Jang, *et al.*, 2000; Plowright, *et al.*, 2000, Ronchetti, *et al.*, 2001). FGFR3 currently appears to be the most frequently mutated oncogene in bladder cancer where it is mutated in almost 50% of the cases and in about 70% of cases having recurrent superficial bladder tumors (Cappellen, *et al.*, 1999; van Rhijn, *et al.*, 2001; 5 Billerey, *et al.*, 2001).

In this context, the consequences of FGFR3 signaling appear to be cell type-specific. In chondrocytes, FGFR3 hyperactivation results in growth inhibition (reviewed in Ornitz, 2001), whereas in the myeloma cell it contributes to tumor progression (Chesi *et al.*, 2001).

Tyrosine Kinase Inhibitors

- 10 In view of the apparent link between PK-related cellular activities and a number of human diseases and disorders, a great deal of effort has been invested to identify ways to modulate PK activity (Cohen, 2002). Some of these attempts have involved biomimetic approaches using large molecules patterned on those involved in the actual cellular processes, e.g., mutant ligands (US 4,966,849) and soluble receptors and antibodies (WO 94/10202).
- 15 In addition, small molecules have been described as tyrosine kinase inhibitors and said to be useful in the treatment of cancer. These include, for example aminopyrazole derivatives (US 5,916,908), pyrazolopyrimidine derivatives (US 5,593,997), pyrrolopyrimidine derivatives (Traxler and Furet, 1999; Traxler *et al.*, 1997; Traxler *et al.*, 1996); US 5,639,757), anilino purine derivatives (WO 00/49018), anilinopyridopyrimidine derivatives 20 (US 6,169,091), anilinopyrimidine derivatives (US 5,958,935), benzoquinazoline derivatives (WO 97/13760), benzothiazole derivatives (WO 99/24035), benzylidene-1,3-dihydro-indol-2-one derivatives (US 6,268,391), benzylphosphonic acid compounds (WO 91/15495, EP 0526488), bis-monocyclic, bicyclic and heterocyclic aryl compounds (WO 92/20642), 1-cyclopropyl-4-pyridylquinolones (US 5,330,992), imidazopyrazine 25 derivatives (US 5,990,109), imidazoquinoxaline derivatives (WO 99/09845, WO 99/10341, US 6,235,740), indolinone derivatives (US 6,130,238, US 6,133,305, US 6,147,106), oxyindolylquinazoline derivatives (WO 99/10349), pyrazolopyrimidine (WO 98/14449, 98/14451), pyridopyrimidine derivatives (WO96/15128, WO 97/13771, WO 98/02437, WO 98/02438, US 5,733,913; US 6,174,889; US 6,207,669), porphyrin and 30 corrole derivatives (WO 00/27379); substituted pyrimidine derivatives (WO 01/00214), quinazoline derivatives (EP 0566266; US 6,399,602), quinoline and quinazoline derivatives (WO 97/03069, WO 98/02434, WO 00/50405, US 5,318,976), styryl compounds (US 5,217,999), styryl-substituted pyridyl compounds (US 5,302,606),

selenaindoles and selenides (PCT 94/03427), thiazolecarboxamide derivatives (WO 00/62778), thiazolo-oxyindole derivatives (WO 99/15500), tricyclic polyhydroxylic compounds (WO 92/21660) and vinylene-azaindole derivatives (WO 94/14808). In addition, Rewcastle and coworkers (1996, 1997, 1998) have described several classes of
5 compounds specific for the EGFR family of RPTKs.

Some of the compounds of general formula (I) disclosed herein have been described in US 6,015,827 and US 6,100,283 where they were reported to potentiate the cytotoxicity of DNA damaging agents such as certain cytotoxic antitumor drugs and radiation used in radiotherapy. Certain compounds of general formula (II) have been disclosed in European
10 Patent publications 0293146, 0357172 and 0370760 and US 5,023,261 and US 5,073,553, where they were reported useful for the prevention or treatment of osteoporosis.

The inventors have now found that the said known compounds, as well as some novel compounds are also effective tyrosine kinase inhibitors useful for the treatment of protein tyrosine kinase related disorders and diseases including angiogenesis, particularly tumor
15 angiogenesis, malignant and non-malignant proliferative diseases and FGFR-related skeletal diseases and disorders.

Citation of any document herein is not intended as an admission that such document is pertinent prior art, or considered material to the patentability of any claim of the present application. Any statement as to content or a date of any document is based on the
20 information available to applicant at the time of filing and does not constitute an admission as to the correctness of such a statement.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide small organic compounds that inhibit aberrant protein tyrosine kinase activity.

It is another object of the present invention to provide a pharmaceutical composition
5 comprising small organic compounds that inhibits aberrant protein tyrosine kinase activity.

It is yet another object of the present invention to provide a method for inhibiting aberrant tyrosine kinase activity in the cells of patients in need thereof.

It is a further object of the present invention to provide a method for inhibiting aberrant receptor protein tyrosine kinase activity in the cells of patients in need thereof.

10 It is yet a further object of the present invention to provide a pharmaceutical composition comprising small organic compounds that inhibits aberrant protein tyrosine kinase activity useful for treating skeletal and proliferative disorders.

It is yet another object of the present invention to provide methods for the use of small organic compounds to prepare medicaments useful for treating and preventing skeletal and
15 proliferative disorders.

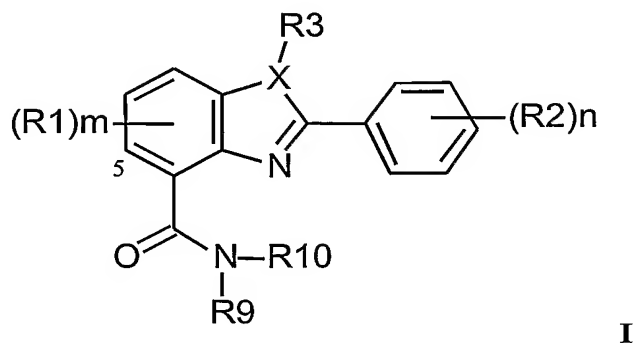
These and other objects are met by the invention disclosed herein.

It has now been found according to the present invention that certain heterocyclic compounds, more particularly certain substituted 2-aryl benzimidazole and benzofuro [3,2-c]quinoline compounds inhibit abnormal protein tyrosine kinase activity and thus can be
20 useful in the treatment of diseases and disorders associated with abnormal protein tyrosine kinase activity.

It is explicit that the present invention excludes known compounds, specifically those disclosed in US 5,023,261; US 5,073,553; US 6,015,827 and US 6,100,283; and European Patent publications 0293146, 0357172 and 0370760, yet certain novel uses of these
25 compounds are now disclosed and claimed.

The present invention thus relates, in one aspect, to a compound selected from the heterocyclic compounds of structural formulae (I) and (II), disclosed below.

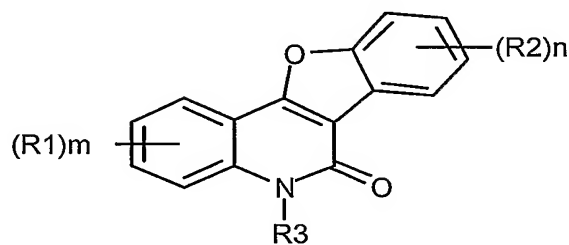
The invention provides novel compounds having the general formula (I):



or a stereoisomer or a pharmaceutically acceptable salt thereof; wherein
X is N or O;

- 5 R1 and R2 are at each occurrence independently selected from halogen, nitro, cyano, trifluoromethyl, hydrocarbyl, OR4, SR4, SOR5, SO₂R5, COOH, COR6, SONR7R8, SO₂NR7R8 and NR7R8;
- R3 is selected from H or R1, and is absent when X is O;
- R9 and R10 are independently selected from H and R1;
- 10 R4 is selected from H, hydrocarbyl, COR6, and CONR7R8;
- R5 is hydrocarbyl;
- R6 is selected from H, hydrocarbyl, OR5 and NR7R8;
- R7 and R8 are each independently selected from H or hydrocarbyl, or one of R7 or R8 is H or hydrocarbyl and the other is COR5, COOR5 or CONR7R8, or R7 and R8 together with
- 15 the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and sulfur;
- m is 0 to 3 and n is 0 to 5;
- with the proviso that a) when m is 0, at least one of R3, R9 or R10 is other than H; and b)
- 20 when X is O, at least one of R9 or R10 is other than H.

The invention further provides novel compounds of the general formula (II):



or a stereoisomer or a pharmaceutically acceptable salt thereof; wherein

R1 and R2 are at each occurrence independently selected from halogen, nitro, cyano,

5 trifluoromethyl, hydrocarbyl, OR4, SR4, SOR5, SO₂R5, COOH, COR6, SONR7R8, SO₂NR7R8 and NR7R8;

R3 is H or R1;

R4 is selected from H, hydrocarbyl, COR6, and CONR7R8;

R5 is hydrocarbyl;

10 R6 is selected from H, hydrocarbyl, OR5 and NR7R8;

R7 and R8 are each independently selected from H or hydrocarbyl, or one of R7 or R8 is H or hydrocarbyl and the other is COR5, COOR5 or CONR7R8, or R7 and R8 together with the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and

15 sulfur;

m and n independently are an integer from 0 to 4;

with the proviso that when R3 is H and m is 0, 1 or 2 and n is 0 or 1, R1 is other than, C₁-C₁₀ alkyloxy, COOH, CONH₂, CO(CH₂)₂₋₇OH, OR4, N-mono-alkylcarbamoyl, N,N-di-alkylcarbamoyl, alkylsulfonyl or CHO.

20 One currently preferred embodiment of the present invention is a compound of general formula (I), wherein X is N, m is 1, R1 at position 5 is radical NHCOCH₃, R9 and R10 are H, R3 is CH₂-CH₂-COOH, CH₂-CH₂-COOR5, or CH₂-CH₂-CONR7R8, wherein R5 is C₁-C₈ alkyl, preferably methyl, and R7 and R8 are each independently selected from H or hydrocarbyl or R7 and R8 together with the nitrogen atom to which they are attached form

25 a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and sulfur, n is 2 and R2 is C₁-C₈ alkoxy, preferably methoxy, most preferably at positions 3 and 5 of the phenyl radical, denoted herein 3-(5-acetylamino-4-carbamoyl-2-(3,5-dimethoxyphenyl)-benzimidazol-1yl)-propionic acid.

One currently more preferred embodiment of the present invention is a compound of general formula (II) denoted herein 3-hydroxy 9-nitro-5H-benzofuro[3,2-c] quinoline-6-one (compound 6). One currently most preferred embodiment of the present invention is a compound of general formula (II) denoted herein 3-methylcarbamoyloxy 9-amino-5H-
5 benzofuro[3,2-c] quinoline-6-one (Compound 8). Position 5 is marked on general formula (I).

According to the principles of the present invention, pharmaceutical compositions, which inhibit protein tyrosine kinase activity, are provided. These compositions are useful in preventing or treating disorders and diseases associated with protein tyrosine kinase
10 activation. In a currently preferred embodiment the protein tyrosine kinase is a receptor protein tyrosine kinase.

One aspect of the present invention provides a pharmaceutical composition comprising as an active agent at least one heterocyclic compound selected from the heterocyclic compounds of general formulae (I) and (II) as described above, or a pharmaceutically
15 acceptable salt thereof, together with a pharmaceutically acceptable carrier or excipient.

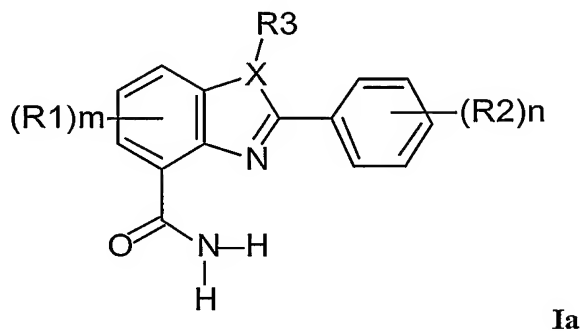
Another aspect of the present invention provides methods for treating or inhibiting the protein tyrosine kinase related diseases and disorders by administering a therapeutically effective amount of a pharmaceutical composition comprising at least one heterocyclic compound selected from the heterocyclic compounds of general formulae (I) and (II) as
20 described above, to a subject in need thereof.

According to another aspect of the present invention, certain known compounds according to general formulae (Ia) and (IIa) are provided for the use in the preparation of a medicament for the prevention or treatment of tyrosine kinase related disorders and diseases.

25

30

The present invention provides the use of a compound of general formula (Ia):



wherein

X is N or O;

- 5 R1 and R2 at each occurrence are independently selected from halogen, nitro, cyano, trifluoromethyl, hydrocarbyl, OR4, COOH, COR6 and NR7R8;

R3 is H or R1, and is absent when X is O;

R4 is selected from H, hydrocarbyl, COR6, and CONR7R8;

R5 is hydrocarbyl;

- 10 R6 is selected from H, hydrocarbyl, OR5 and NR7R8;

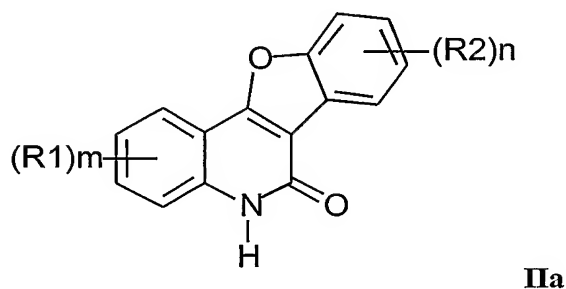
R7 and R8 are each independently selected from H or hydrocarbyl, or one of R7 or R8 is H or hydrocarbyl and the other is COR5, COOR5 or CONR7R8, or R7 and R8 together with the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and

- 15 sulfur; and

m is 0 to 3 and n is 0 to 5;

for the preparation of a medicament for treating or preventing protein tyrosine kinase related diseases and disorders.

The present invention further provides the use of a compound of general formula (IIa):



wherein

R1 and R2 are each independently selected from halogen, hydrocarbonyl, OR4, SR4, SOR5,
 5 SO₂R5, COOH, COR6, SONR7R8, SO₂NR7R8 and NR7R8;

R4 is selected from H, hydrocarbonyl, COR6, and CONR7R8;

R5 is hydrocarbonyl;

R6 is selected from H, hydrocarbonyl, OR5 and NR7R8;

R7 and R8 are each independently selected from H or hydrocarbonyl, or one of R7 or R8 is H
 10 or hydrocarbonyl and the other is COR5, COOR5 or CONR7R8, or R7 and R8 together with
 the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic
 ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and
 sulfur; and

m and n are independently selected from an integer of 0 to 4;

15 for the preparation of a medicament for treating or preventing protein tyrosine kinase
 related diseases and disorders.

According to another aspect of the present invention, certain known compounds according
 to general formulae (Ia) and (IIa) are provided for the use in the preparation of a
 medicament for inhibiting abnormal protein kinase activity.

20 In another aspect, the present invention provides a method for inhibiting abnormal protein
 tyrosine kinase (PTK) activity, particularly receptor protein tyrosine kinase (RPTK)
 activity, which comprises administering to an individual in need thereof a PTK inhibitor
 selected from at least one heterocyclic compound selected from the heterocyclic
 compounds of structural formulae I and II, (Ia) and (IIa) wherein said compound is
 25 administered in an amount sufficient to inhibit said PTK, e.g. RPTK, activity.

In still a further aspect, the present invention provides novel conjugates of the compounds of the structural formulae I, II, (Ia) and (IIa) above with targeting moieties such as peptides, antibodies, growth factors or any other moiety capable of specifically targeting said compound to the protein tyrosine kinase of interest.

- 5 In a currently preferred embodiment, the pharmaceutical compositions of the present invention may be used for treating or preventing diseases and disorders associated with aberrant protein tyrosine kinase activity, including angiogenesis, proliferative disorders including benign and malignant tumors, and FGFR related skeletal diseases.

In a non-limiting example, EGFR/ErbB1 is associated with fibrosarcomas, mammary
10 carcinoma, glioblastoma multiforme, ovarian, non-small-cell lung and other cancers; ErbB2/HER2/Neu is associated with mammary, ovarian, gastric, non-small-cell lung and colon cancers; ErbB3/HER3 is associated with mammary cancer; ErbB4/HER4 is associated with mammary carcinoma and granulose cell tumors; IGF-1R is associated with cervical and other cancers; Flk2/FLT3 is associated with hematopoietic malignancies;
15 Flk/VEGFR1, Flk1/VEGFR2 and Flt4/VEGFR3 are associated with tumor angiogenesis; FGFR1 is associated with acute myelogenous leukemia (AML) and with various tumors, and point mutations thereof are associated with autosomal skeletal disorders/dysplasias; FGFR2/K-SAM is associated with gastric, mammary and prostate carcinomas and certain types of craniosynostosis and skeletal dysplasias; FGFR3 is associated with multiple
20 myeloma, transitional cell carcinoma, cervical and breast cancer and point mutations thereof are associated with skeletal dysplasias such as achondroplasia, thanatophoric dysplasia and hypochondroplasia; FGFR4 is associated with mammary, pituitary and ovarian cancers; TrkA and HGFR are associated with papillary thyroid carcinomas.

BRIEF DESCRIPTION OF THE FIGURES

- 25 **Figure 1** is a graph showing the effect of the compound **2** on the proliferation of FDCP cells transfected with human wild type FGFR3 (R3 cells) or FGFR1 (R1 cells) grown in the presence of either FGF9 (F9) or IL-3.

Figure 2 is a Western blot showing the inhibition of FGFR3 and JNK phosphorylation by Compound **2**.

- 30 **Figure 3** is a graph showing the effect of Compound **2** on G369C achondroplasia heterozygote mice femora growth in organ culture.

Figure 4 is a graph showing the effect of Compound **2** on the growth rate of G369C achondroplasia heterozygote mice femora bones.

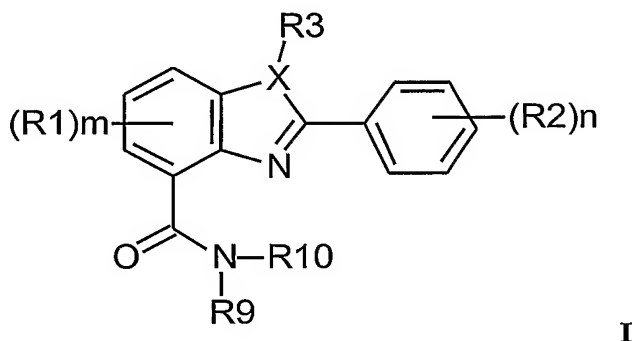
Figure 5 presents a table of the results of an in vivo proliferation assay using certain compounds of the present invention.

- 5 **Figures 6 and 7** show the synthetic pathways of certain embodiments of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides novel heterocyclic compounds and pharmaceutical compositions comprising at least one said compound useful as protein tyrosine kinase inhibitors. The novel compounds of the present invention are compounds of general formulae I and II. Furthermore, certain known compounds having the general formulae (Ia) and (IIa) may be used to prepare medicaments effective in the treatment of tyrosine kinase related diseases and disorders.

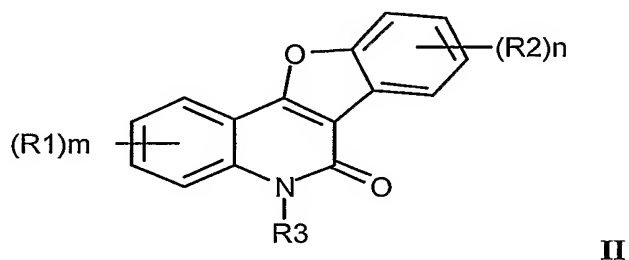
The invention provides a novel compound having the general formula (I):



- or a stereoisomer or a pharmaceutically acceptable salt thereof; wherein
- X is N or O;
- R1 and R2 are at each occurrence independently selected from halogen, nitro, cyano, trifluoromethyl, hydrocarbyl, OR4, SR4, SOR5, SO₂R5, COOH, COR6, SONR7R8, SO₂NR7R8 and NR7R8;
- R3 is selected from H or R1, and is absent when X is O;
- R9 and R10 are independently selected from H and R1;
- R4 is selected from H, hydrocarbyl, COR6, and CONR7R8;
- R5 is hydrocarbyl;
- R6 is selected from H, hydrocarbyl, OR5 and NR7R8;
- R7 and R8 are each independently selected from H or hydrocarbyl, or one of R7 or R8 is H or hydrocarbyl and the other is COR5, COOR5 or CONR7R8, or R7 and R8 together with the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and sulfur;
- m is 0 to 3 and n is 0 to 5;

with the proviso that a) when m is 0, at least one of R3, R9 or R10 is other than H; and b) when X is O, least one of R3, R9 or R10 is other than H.

The invention further provides novel compounds of the general formula (II):



- 5 or a stereoisomer or a pharmaceutically acceptable salt thereof; wherein
 R1 and R2 are at each occurrence independently selected from halogen, nitro, cyano, trifluoromethyl, hydrocarbyl, OR4, SR4, SOR5, SO₂R5, COOH, COR6, SONR7R8, SO₂NR7R8 and NR7R8;
 R3 is H or R1;
- 10 R4 is selected from H, hydrocarbyl, COR6, and CONR7R8;
 R5 is hydrocarbyl;
 R6 is selected from H, hydrocarbyl, OR5 and NR7R8;
 R7 and R8 are each independently selected from H or hydrocarbyl, or one of R7 or R8 is H or hydrocarbyl and the other is COR5, COOR5 or CONR7R8, or R7 and R8 together with
 15 the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and sulfur;
 m and n independently are an integer from 0 to 4;
 with the proviso that when R3 is H and m is 0, 1 or 2 and n is 0 or 1, R1 is other than
 20 C₁-C₁₀ alkyloxy, COOH, CONH₂, CO(CH₂)₂₋₇OH, OR4, N-mono-alkylcarbamoyl, N,N-di-alkylcarbamoyl, alkylsulfonyl or CHO.

One aspect of the present invention provides a pharmaceutical composition comprising as an active agent at least one heterocyclic compound selected from the heterocyclic compounds of general formulae (I) and (II) as described above, or a pharmaceutically
 25 acceptable salt thereof, together with a pharmaceutically acceptable carrier or excipient.

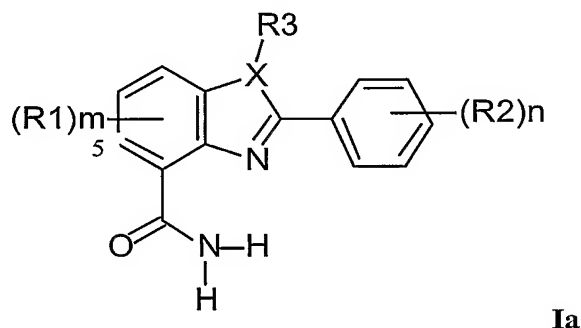
Another aspect of the present invention provides methods for treating or inhibiting the protein tyrosine kinase related diseases and disorders by administering a therapeutically effective amount of a pharmaceutical composition comprising at least one heterocyclic

compound selected from the heterocyclic compounds of general formulae (I) and (II) as described above, to a subject in need thereof.

According to another aspect of the present invention, certain known compounds according to general formulae (Ia) and (IIa) are now disclosed to be inhibitors of protein tyrosine

5 kinase and are useful in the preparation of a medicament for inhibiting abnormal protein kinase activity.

The present invention provides the use of a compound of general formula (Ia):



wherein

10 X is N or O;

R1 and R2 at each occurrence are independently selected from halogen, nitro, cyano, trifluoromethyl, hydrocarbyl, OR4, COOH, COR6 and NR7R8;

R3 is H or R1, and is absent when X is O;

R4 is selected from H, hydrocarbonyl, COR6, and CONR7R8;

15 R5 is hydrocarbyl;

R6 is selected from H, hydrocarbonyl, OR5 and NR7R8;

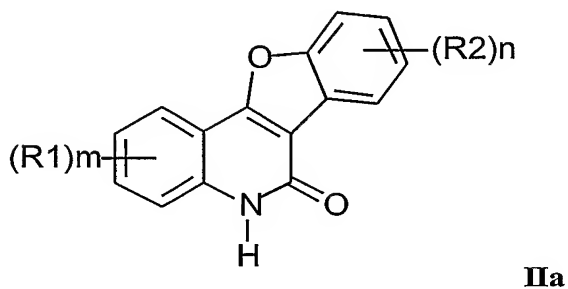
R7 and R8 are each independently selected from H or hydrocarbyl, or one of R7 or R8 is H or hydrocarbyl and the other is COR5, COOR5 or CONR7R8, or R7 and R8 together with the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and sulfur; and

20 ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and sulfur; and

m is 0 to 3 and n is 0 to 5;

for the preparation of a medicament for treating or preventing protein tyrosine kinase related diseases and disorders.

The present invention further provides the use of a compound of general formula (IIa):



wherein

R1 and R2 are each independently selected from halogen, hydrocarbyl, OR4, SR4, SOR5,
5 SO₂R5, COOH, COR6, SONR7R8, SO₂NR7R8 and NR7R8;

R4 is selected from H, hydrocarbyl, COR6, and CONR7R8;

R5 is hydrocarbyl;

R6 is selected from H, hydrocarbyl, OR5 and NR7R8;

R7 and R8 are each independently selected from H or hydrocarbyl, or one of R7 or R8 is H
10 or hydrocarbyl and the other is COR5, COOR5 or CONR7R8, or R7 and R8 together with
the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic
ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and
sulfur; and

m and n are independently selected from an integer of 0 to 4;

15 for the preparation of a medicament for treating or preventing protein tyrosine kinase
related diseases and disorders.

For convenience and clarity certain terms employed in the specification, examples and
claims are described herein.

Abbreviations: **Axl**, a Tyro3 PTK; **bFGF**, basic fibroblast growth factor; **CSF1-R**,
20 colony-stimulating factor-1 receptor; **EGF**, epidermal growth factor; **EGFR**, epidermal
growth factor receptor; **EphR**, ephrin receptor; **erbB2,3,4**, members of the EGFR family;
FGF, fibroblast growth factor; **FGFR**, FGF receptor; **FGFR1**, FGF receptor-1; **FGFR2**,
FGF receptor-2; **FGFR3**, FGF receptor-3; **HB-EGF**, heparin-binding EGF-like growth
factor; **HGFR**, hepatocyte growth factor receptor; **IGF-1R**, insulin growth factor 1
25 receptor; **InsR**, insulin receptor; **LTK**, leukocyte tyrosine kinase; **NGFR**, nerve growth

factor receptor; **PDGFR**, platelet-derived growth factor receptor; **PK**, protein kinase; **PTK**, protein tyrosine kinase; **RET**, rearranged during transfection; **ROS**, RPTK expressed in some epithelial cell types; **RPTK**, receptor protein tyrosine kinase; **SCFR**, stem cell factor receptor; **TIE**, tyrosine kinase receptor in endothelial cells; **VEGFR**,
 5 vascular endothelial growth factor receptor; **NMR**, nuclear magnetic resonance; **EI**, electron ionization; **XTT**, 2,3-bis-(2-methoxy-4-nitro- 5-sulfophenyl)-2H-tetrazolium-5-carboxanilide, Na₂.

As used herein, the term "halo" or "halogen" includes fluoro, chloro, bromo and iodo.

The term "hydrocarbyl" in any of the definitions of the different radicals R1-R8 includes
 10 any radical containing carbon and hydrogen including saturated or unsaturated, aromatic, straight or branched chain or cyclic including polycyclic, such as, but not being limited to, C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₁₀ cycloalkyl, aryl such as phenyl and naphthyl, ar(C₁-C₈)alkyl such as benzyl, and any such hydrocarbyl radical substituted by one or more radicals R1 as defined above.

15 The terms "C₂ -C₈ alkenyl" and "C₂ -C₈ alkynyl " typically mean straight and branched hydrocarbon radicals having 2-8 carbon atoms and 1 double or triple bond, respectively, and include ethenyl, 3-buten-1-yl, 2-ethenylbutyl, 3-octen-1-yl, and the like, and propynyl, 2-butyne-1-yl, 3-pentyne-1-yl, and the like. C₂-C₆ alkenyl radicals are preferred. The term "C₃ -C₁₀ cycloalkyl" means a cyclic or bicyclic hydrocarbyl group such as cyclopropyl,
 20 cyclobutyl, cyclopentyl, cyclohexyl, adamantyl, bicyclo[3.2.1]octyl, bicyclo[2.2.1]heptyl, and the like.

The term "C₁ -C₈ alkyl" typically means a straight or branched hydrocarbon radical having 1-8 carbon atoms and includes, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, n-pentyl, 2,2-dimethylpropyl, n-hexyl, n-heptyl, n-octyl, and
 25 the like. Preferred are C₁ -C₆ alkyl groups, also denoted herein "lower alkyl".

When one of the radicals R1, R2, R3 or R6 is an alkoxy radical, the term means preferably a "C₁ -C₈ alkoxy" radical including methoxy, ethoxy, isopropoxy, tert-butoxy, n-octyloxy, and the like. C₁ -C₆ alkoxy groups, also denoted herein "lower alkoxy" are preferred.

Typical "C₁ -C₈ alkanoyl" groups formed by COR6 radicals include formyl, acetyl,
 30 propionyl, butyryl, and isobutyryl, and typical "C₁-C₈ alkanoyloxy" groups formed by OCOR6 radicals include acetoxyl, tert-butanoyloxy, pentanoyloxy, and the like. Similar

radicals are contemplated for the corresponding groups having an S atom such as SR₄, SOR₅, and SO₂R₅.

In the groups NR₇R₈, CONR₇R₈, SONR₇R₈, SO₂NR₇R₈, or when any of the alkyl, alkenyl, alkynyl and alkanoyl groups are substituted with NR₇R₈, R₇ and R₈ are each H or hydrocarbyl as defined above or form together with the N atom to which they are attached a saturated or unsaturated, preferably a 5- or 6-membered, heterocyclic ring, optionally containing 1 or 2 further heteroatoms selected from nitrogen, oxygen, and sulfur. Such rings may be substituted, for example with one or two C₁-C₆ alkyl groups. Examples of radicals NR₇R₈ include, without being limited to, amino, dimethylamino, diethylamino, ethylmethylamino, dimethylaminomethyl, phenylmethylamino, pyrrolidino, piperidino, tetrahydropyridino, piperazino, morpholino, thiazolino, and the like, and examples of radicals hydrocarbyl substituted by NR₇R₈ include 4-diethylamino-3-buten-1-yl, 5-ethylmethylamino-3-pentyn-1-yl, 4-morpholinobutyl, 4-(4-methylpiperazin-1-yl)butyl, 4-tetrahydropyridinylbutyl-, 2-methyltetrahydropyridinomethyl-, 3-imidazolidin-1-yl-propyl, 4-tetrahydrothiazol-3-yl-butyl, and the like.

The terms "N-mono-alkylcarbamoyl" and "N,N-di-alkylcarbamoyl" as used herein refer to -OCONHR and -OCONR₂ respectively, wherein R represents a C₁-C₆ alkyl group.

Where any of the compounds referred to can exist in more than one enantiomeric form, all such forms and mixtures thereof as well as uses thereof are within the scope of the invention.

In one preferred embodiment, the compound is a substituted 2-aryl-benzimidazole-4-carboxamide compound of the structural formula I.

According to this embodiment, the compound is of the structural formula I wherein X is N, namely a substituted 2-aryl-benzimidazole-4-carboxamide derivative, wherein:

R₁ is each independently selected from C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₁-C₈ alkanoyl, OR₄, NR₇R₈, NHCONR₇R₈, NHCOOR₅, NR₅CONR₇R₈, NR₅COOR₅, OCONR₇R₈, OCOOR₅, wherein R₄, R₅, R₇ and R₈ are each independently selected from hydrogen or C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₁₀ cycloalkyl, and C₁-C₈ alkanoyl optionally substituted by OR₄, COOH, COOR₅, or NR₇R₈, wherein R₇ and R₈ may also together with the nitrogen atom to which they are attached form a 5- or 6-membered heterocyclic ring optionally containing 1 or 2 further heteroatoms selected from nitrogen, oxygen, and sulfur; and m is 0 to 3;

R2 is each independently selected from halo, nitro, trifluoromethyl, optionally substituted phenyl, cyano, COOH, COOR5, COR6, SO₂R5, C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, OH, C₁-C₈ alkoxy, SH, S-C₁-C₈ alkyl, C₁-C₈ alkanoyl, C₁-C₈ alkanoyloxy, or NR7R8 and wherein the alkyl, alkenyl, alkynyl, alkoxy and alkanoyl groups may be substituted by

5 NR7R8, where R7 and R8 are as defined above, and n is 0 to 5; and

R3, R9 and R10 and are each independently selected from hydrogen, C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, NHCONR7R8, NHCOOR5, NR5CONR7R8, NR5COOR5, OR4, OCONR7R8, C₁-C₈ alkanoyl, C₁-C₈ alkylamino or di-C₁-C₈ alkylamino; and wherein the alkyl, alkenyl, alkynyl and alkanoyl groups may be substituted by COOH, COOR5,

10 CONR7R8 or NR7R8, where R4, R5, R7 and R8 are as defined above.

It is explicit that the present invention excludes known compounds of general formula (I), specifically those disclosed in US 6,015,827 and US 6,100,283.

In one preferred embodiment, the compound is of formula (I) wherein X is N, m is 1, R1 at position 5 is a radical NHCOCH₃, R9 and R10 are H, R3 is CH₂-CH₂-COOH, CH₂-CH₂-

15 COOR5, or CH₂-CH₂-CONR7R8, wherein R5 is C₁-C₈ alkyl, preferably methyl, and R7 and R8 are each independently selected from H or hydrocarbyl or R7 and R8 together with the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and sulfur, n is 2 and R2 is C₁-C₈ alkoxy, preferably methoxy, most preferably at positions 3

20 and 5 of the phenyl radical. A currently more preferred embodiment of the present invention is denoted herein 3-(5-acetylamino-4-carbamoyl-2-(3,5-dimethoxyphenyl)-benzimidazol-1yl)-propionic acid.

In another preferred embodiment, the compound is a substituted benzofuro[3,2-c]quinoline compound of the structural formula (II). Preferably, said composition comprises a

25 compound of formula (II), wherein:

R1 is each independently selected from C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₁-C₈ alkanoyl, OR4, NR7R8, NHCONR7R8, NHCOOR5, NR5CONR7R8, NR5COOR5, OCONR7R8, OCOOR5, wherein R4, R5, R7 and R8 are each independently selected from hydrogen or C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₁₀ cycloalkyl, and C₁-C₈

30 alkanoyl optionally substituted by OR4, COOH, COOR5, or NR7R8, wherein R7 and R8 may also together with the nitrogen atom to which they are attached form a 5- or 6-

membered heterocyclic ring optionally containing 1 or 2 further heteroatoms selected from nitrogen, oxygen, and sulfur; and m is 0 to 4;

R2 is each independently selected from halo, nitro, trifluoromethyl, optionally substituted phenyl, cyano, COOH, COOR5, COR6, SO2R5, C1-C8 alkyl, C2-C8 alkenyl, C2-C8 alkynyl, OH, C1-C8 alkoxy, SH, S-C1-C8 alkyl, C1-C8 alkanoyl, C1-C8 alkanoyloxy, or NR7R8 and wherein the alkyl, alkenyl, alkynyl, alkoxy and alkanoyl groups may be substituted by NR7R8, where R7 and R8 are as defined above, and n is 0 to 4; and

R3 is selected from hydrogen, C1-C8 alkyl, C2-C8 alkenyl, C2-C8 alkynyl, NHCONR7R8, NHCOOR5, NR5CONR7R8, NR5COOR5, OR4, OCONR7R8, C1-C8 alkanoyl, C1-C8 alkylamino or di-C1-C8 alkylamino; and wherein the alkyl, alkenyl, alkynyl and alkanoyl groups may be substituted by COOH, COOR5, CONR7R8 or NR7R8, wherein R4, R5, R7 and R8 are as defined above.

It is explicit that the present invention excludes known compounds of general formula (II), specifically those disclosed in US 5,023,261; US 5,073,553; and European Patent publications 0293146, 0357172 and 0370760.

According to one currently preferred embodiment R3 is H, m is 1, R1 is OH, n is 1, R2 is NO2, herein denoted 3-hydroxy 9-nitro-5H-benzofuro[3,2-c] quinoline-6-one (compound 6).

According to one currently more preferred embodiment R3 is H, m is 1, R1 is dimethyl carbamoyl, n is 1, R2 is NH2, herein denoted 3-methylcarbamoyloxy 9-amino-5H-benzofuro[3,2-c] quinoline-6-one (compound 8).

Where any of the compounds referred to can exist in more than one enantiomeric form, all such forms, mixtures thereof, preparation and uses are within the scope of the invention.

According to one currently preferred embodiment, the pharmaceutical composition comprises as an active ingredient the compound of general formula (I) denoted 3-(5-acetylamino-4-carbamoyl-2-(3,5-dimethoxyphenyl)-benzimidazol-1yl)-propionic acid, and a pharmaceutically acceptable carrier or diluent.

According to one currently more preferred embodiment, the pharmaceutical composition comprises as an active ingredient the compound of general formula (II) denoted 3-hydroxy 9-nitro-5H-benzofuro[3,2-c] quinoline-6-one, and a pharmaceutically acceptable carrier or diluent.

According to one currently most preferred embodiment, the pharmaceutical composition comprises as an active ingredient the compound of general formula (II) denoted 3-methylcarbamoyloxy 9-amino-5H-benzofuro[3,2-c]quinoline-6-one, and a pharmaceutically acceptable carrier or diluent.

- 5 In one currently preferred embodiment of the present invention it is now disclosed that certain known 2-aryl-benzimidazole-4-carboxamide and benzofuro[3,2-c]quinoline compounds of general formulae (Ia) and (IIa), respectively, may be used to prepare medicaments for the treatment of protein tyrosine kinase related disorders and diseases.

In one embodiment, a benzofuro[3,2-c]quinoline compound of the structural formula (II) wherein R₃ is hydrogen is useful for the preparation of a medicament. In another embodiment, a compound of formula (II) wherein R₁ and R₂ are OR₄, R₄ is hydrogen or OCONR₇R₈, R₇ and R₈ are each C₁-C₃ alkyl, R₃ is H, and each of m and n is 1 is disclosed. In further embodiments, a benzofuro[3,2-c]quinoline compound of the structural formula II herein identified as Compound 1, in which R₃ is hydrogen, and R₁ and R₂ are both OH; or the compound herein identified as Compound 2, in which R₃ is hydrogen, and R₁ and R₂ are both OCON(CH₃)₂; or the compound herein identified as Compound 3, in which R₃ is hydrogen, and R₁ and R₂ are both OCON(C₂H₅)₂; or the compound herein identified as Compound 4, in which R₃ is hydrogen, and R₁ and R₂ are both OCON(i-C₃H₇)₂ are now disclosed for use in the preparation of a medicament for treating protein tyrosine kinase associated diseases and disorders.

Compounds of formula I wherein X is O and m is 0, or X is N and m is 0, have been disclosed in US 6,015,827 and US 6,100,283, respectively, each of said patents herein incorporated by reference in its entirety as if fully disclosed herein, as potent inhibitors of the DNA repair enzyme poly(ADP-ribose)polymerase or PARP enzyme, and are said to be useful therapeutic compounds for use in conjunction with DNA-damaging cytotoxic drugs or radiotherapy.

The compounds of formula (II) wherein R₃ is H and R₁ and R₂ are OR₄ have been disclosed in European Patent publications 0293146, 0357172 and 0370760 and US 5,023,261 and US 5,073,553, each of said patents herein incorporated by reference in its entirety as if fully disclosed herein, and are said to be useful as anti-osteoporosis agents.

According to one aspect of the present invention provided is the use of a compound according to formula Ia for the preparation of a medicament for inhibiting abnormal PTK wherein m is 1 and X is N or O.

According to another aspect of the present invention provided is the use of a compound
5 according to formula Ia for the preparation of a medicament for inhibiting abnormal PTK wherein R3 is hydrogen, R1 and R2 are OR4, wherein R4 is hydrogen or OCONR7R8 wherein R7 and R8 are each C₁-C₃ alkyl, and each of m and n is 1.

A currently preferred embodiment of the present invention is the use of a compound according to formula Ia for the preparation of a medicament for inhibiting abnormal PTK,
10 wherein R3 is H and n is 1 and R1 and R2 are OH or OCON(CH₃)₂.

The compounds of formula (I) may be prepared by the methods described in the above-mentioned US 6,100, 283 and US 6,015,827, or by the syntheses outlined herein, which describes a typical method for the preparation of 3-(5-acetylamino-4-carbamoyl-2-phenylbenzoimidazol-1-yl)-propionic acid from commercially available dinitrophthalic anhydride
15 (modified from (White, 2000)).

Compounds of Formula (II) may be prepared according to the syntheses outlined in Scheme 2 herein, which describes the synthesis of the compounds hereinafter identified as Compounds 1 and 2 from commercially available anisidine (EP Publication No. 357172).

Although Schemes 1 and 2 indicate exact structures, the methods apply widely to
20 analogous compounds of formula (I) or formula (II), given appropriate consideration to protection and deprotection of reactive functional groups by methods standard to the art of organic chemistry. For example, hydroxy groups, in order to prevent unwanted side reactions, generally need to be converted to ethers or esters during chemical reactions at other sites in the molecule. The hydroxy protecting group is readily removed to provide the
25 free hydroxy group. Amino groups and carboxylic acid groups are similarly derivatized to protect them against unwanted side reactions. Typical protecting groups, and methods for attaching and cleaving them, are described fully by Greene and Wuts in Protective Groups in Organic Synthesis, John Wiley and Sons, New York, (2nd Ed, 1991), and McOmie, Protective Groups in Organic Chemistry, Plenum Press, New York, 1973.

30 The compounds of the present invention can exist both in unsolvated and solvated forms, including hydrated forms. In general, the solvated forms, including hydrated forms, are

equivalent to unsolvated forms and are intended to be encompassed within the scope of the present invention.

Currently preferred embodiments of the present invention include pharmaceutical compositions comprising the compounds of structural formulae (I) and (II) above are for
5 use as active ingredients of pharmaceutical compositions useful for inhibiting abnormal tyrosine kinase activity.

Pharmacology

Also contemplated by the present invention are pharmaceutically acceptable salts of the compounds I and II, both salts formed by any carboxy group present in the molecule and a
10 base as well as acid addition and/or base salts.

Pharmaceutically acceptable acid addition salts of the compounds include salts derived from inorganic acids such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydriodic, phosphorous, and the like, as well as the salts derived from organic acids, such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanolic acids, hydroxy
15 alkanolic acids, alkanedioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, etc. Such salts thus include sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, nitrate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, caprylate, isobutyrate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, mandelate, benzoate, chlorobenzoate,
20 methylbenzoate, dinitrobenzoate, phthalate, benzenesulfonate, toluenesulfonate, phenylacetate, citrate, lactate, maleate, tartrate, methanesulfonate, and the like. Also contemplated are salts of amino acids such as arginate and the like and gluconate, galacturonate (see, for example, Berge S. M., et al., "Pharmaceutical Salts," J. of Pharmaceutical Science, 66:1-19 (1977)).

25 The acid addition salts of said basic compounds are prepared by contacting the free base form with a sufficient amount of the desired acid to produce the salt in the conventional manner. The free base form may be regenerated by contacting the salt form with a base and isolating the free base in the conventional manner. The free base forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar
30 solvents, but otherwise the salts are equivalent to their respective free base for purposes of the present invention.

Pharmaceutically acceptable base addition salts are formed with metals or amines, such as alkali and alkaline earth metals or organic amines. Examples of metals used as cations are sodium, potassium, magnesium, calcium, and the like. Examples of suitable amines are N,N'-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, 5 N-methylglucamine, and procaine (see, for example, Berge S. M., et al., "Pharmaceutical Salts," J. of Pharmaceutical Science, 66:1-19 (1977)).

The base addition salts of said acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid 10 and isolating the free acid in the conventional manner. The free acid forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present invention.

The present invention further provides pharmaceutical formulations comprising at least one 15 compound of (I) or (II) together with a pharmaceutically acceptable carrier, diluent, or excipient therefor. The formulations of this invention preferably contain from about 5% to about 70% of the active compound.

The pharmaceutical composition of this invention may be administered by any suitable means, such as orally, intranasally, subcutaneously (SC), intramuscularly (IM), 20 intravenously (IV), intra-arterially, intralesionally or parenterally. Ordinarily, intravenous or oral administration will be preferred.

It will be recognized to those skilled in the art that the following dosage forms may comprise as an active component either a compound of Formula (I) or (II) or a corresponding pharmaceutically acceptable salt or solvate thereof

25 For preparing pharmaceutical compositions with the compounds of the present invention, pharmaceutically acceptable carriers can be either solid or liquid (Pillai and Panchagnula, 2001). Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more substances which may also act as diluents, flavoring agents, binders, preservatives, tablet 30 disintegrating agents, or an encapsulating material. In powders, the carrier is a finely divided solid such as talc or starch which is in a mixture with the finely divided active

component. In tablets, the active component is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.

Suitable carriers include magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium

- 5 carboxymethylcellulose, a low melting wax, cocoa butter, and the like. A preferred form for oral use is capsules, which include the formulation of the active compound with encapsulating material as a carrier providing a capsule in which the active component with or without other carriers, is surrounded by a carrier, which is thus in association with it. Similarly, cachets and lozenges are included. Tablets, powders, capsules, pills, cachets, and
10 lozenges can be used as solid dosage forms suitable for oral administration.

For preparing suppositories, a low melting wax, such as a mixture of fatty acid glycerides or cocoa butter, is first melted and the active component is dispersed homogeneously therein, as by stirring. The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool, and thereby to solidify.

- 15 Liquid form preparations include solutions, suspensions, and emulsions, for example, water or water-propylene glycol solutions. For parenteral injection, liquid preparations can be formulated in solution in aqueous polyethylene glycol solution, isotonic saline, 5% aqueous glucose, and the like. Aqueous solutions suitable for oral use can be prepared by dissolving the active component in water and adding suitable colorants, flavors, stabilizing
20 and thickening agents as desired. Aqueous suspensions suitable for oral use can be made by dispersing the finely divided active component in water with a viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well-known suspending agents.

- Also encompassed by the present invention are solid form preparations which are intended
25 to be converted, shortly before use, to liquid form preparations for oral administration. Such liquid forms include solutions, suspensions, and emulsions.

- The pharmaceutical formulations of the invention may contain, in addition to the active component, colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like. Waxes, polymers, and the like can
30 be utilized to prepare sustained-release dosage forms. In addition, osmotic pumps can be employed to deliver the active compound uniformly over a prolonged period.

The pharmaceutical preparations of the invention are preferably in unit dosage form. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and
5 powders in vials or ampoules. In addition, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

It will be apparent to those of ordinary skill in the art that the therapeutically effective amount of the molecule according to the present invention will depend, *inter alia* upon the
10 administration schedule, the unit dose of molecule administered, whether the molecule is administered in combination with other therapeutic agents, the immune status and health of the patient, the therapeutic activity of the molecule administered and the judgment of the treating physician. As used herein, a “therapeutically effective amount” refers to the amount of a compound required to alleviate one or more symptoms associated with a
15 disorder being treated over a period of time.

The present invention further relates to a method for inhibiting abnormal PTK activity, particularly RPTK activity, which comprises administering to an individual in need thereof a PTK activity inhibitor selected from at least one heterocyclic compound selected from the heterocyclic compounds of structural formulae I and II.

20 Tyrosine Kinase Related Disease

The role of protein tyrosine kinases in the development and progression of tumorigenesis is well documented.

As described by Blume-Jensen and Hunter, 2001, RPTKs are a subclass of transmembrane-spanning receptors endowed with intrinsic, ligand-stimulatable PTK activity. When
25 mutated, overexpressed or altered structurally, RPTKs can become potent oncoproteins, causing cellular transformation. More than 90 PTK genes are known in the human genome: 58 encode transmembrane RPTKs distributed into 20 subfamilies, and 32 encode cytoplasmic, non-receptor PTKs in 10 subfamilies. Of the about 30 tumor-suppressor genes and more than 100 dominant oncogenes known to date, protein kinases, in particular
30 PTKs, comprise a large fraction.

A number of RPTKs and cytoplasmic PTKs are described in Blume-Jensen and Hunter, 2001, and inhibition of abnormal activity of all of them are encompassed by the present

invention. Table 1 of said publication discloses human RPTKs: the prototypic receptor for each family is indicated above the receptor, and the known members are listed below. For example, EGFR/ErbB1, ErbB2/HER2/Neu, ErbB3/HER3 and ErbB4/HER4 are members of the EGFR family; IGF-1R is a member of the insulin receptor (InsR) family; PDGFR- α , PDGFR- β , CSF-1R, kit/SCFR and Flk2/FLT3 are members of the PDGFR family; Flk/VEGFR1, Flk1/VEGFR2 and Flt4/VEGFR3 are members of the VEGFR family; FGFR1, FGFR2/K-SAM, FGFR3, FGFR4 and FGFR5 are members of the FGFR family; TrkA and TrkC are members of the NGFR family; RON is a member of the HGFR family; EphA2, EphB2 and EphB4 are members of the EphR family; Axl is a member of the AXL family; TIE/TIE1 and Tek/TIE2 are members of the TIE family; Ret is a member of the RET family; ROS is a member of the ROS family; and Alk is a member of the LTK family. All these RPTKs are involved in human malignancies. For the meaning of the abbreviations, see Section "Abbreviations" on page 1 of the present specification. The slash between abbreviations indicates that the receptor is known by either one of the designations.

In addition to the RPTKs, Blume-Jensen and Hunter, 2001, describe also human cytoplasmic PTKs in Fig. 2 therein, from which the following are involved in human malignancies: Fgr, Src, Yes1, Lck – members of the SRC family; Abl1, Arg – members of the ABL family; Jak1, Jak2, Jak3 – members of the JAK family; Fak, Pyk2 – members of the FAK family; Fes – member of the FES family; Brk – member of the FRK family; and Syk – member of the SYK family.

The association between abnormal activity of each of the above-mentioned human RPTKs and cytoplasmic PTKs and diseases or disorders, particularly tumor/cancer types, are disclosed in Table 1 of Blume-Jensen and Hunter, 2001, herein incorporated by reference. For example, EGFR/ErbB1 is associated with fibrosarcomas, mammary carcinoma, glioblastoma multiforme, ovarian, non-small-cell lung and other cancers; ErbB2/HER2/Neu is associated with mammary, ovarian, gastric, non-small-cell lung and colon cancers; ErbB3/HER3 is associated with mammary cancer; ErbB4/HER4 is associated with mammary carcinoma and granulose cell tumors; IGF-1R is associated with cervical and other cancers; Flk2/FLT3 is associated with hematopoietic malignancies; Flk/VEGFR1, Flk1/VEGFR2 and Flt4/VEGFR3 are associated with tumor angiogenesis; FGFR1 is associated with acute myelogenous leukemia (AML) and with various tumors, and point mutations thereof are associated with autosomal skeletal

disorders/dysplasias; FGFR2/K-SAM is associated with gastric, mammary and prostate carcinomas, and certain craniosynostosis and skeletal diseases; FGFR3 is associated with multiple myeloma, and point mutations thereof are associated with skeletal dysplasias such as achondroplasia, thanatophoric dysplasia and hypochondroplasia; FGFR4 is associated with mammary, pituitary and ovarian cancers; TrkA and HGFR are associated with papillary thyroid carcinomas. Several of the FGFRs have been shown to be associated with hematopoietic disease (Moroni, et al, 2002)

The heterocyclic compounds of structural formulae (I) and (II) are useful for inhibition of abnormal PTK activity, particularly inhibition of abnormal activity of a RPTK selected from the group consisting of EGFR/ErbB1, ErbB2/HER2/Neu, ErbB/HER3, ErbB4/HER4, IGF-1R, PDGFR- α , PDGFR- β , CSF-1R, kit/SCFR, Flk2/FLT3, Flk/VEGFR1, Flk1/VEGFR2, Flt4/VEGFR3, FGFR1, FGFR2/K-SAM, FGFR3, FGFR4, TrkA, TrkC, HGFR, RON, EphA2, EphB2, EphB4, Axl, TIE/TIE1, Tek/TIE2, Ret, ROS and Alk, as well as for inhibition of abnormal activity of a cytoplasmic PTK selected from the group consisting of Src, Fgr, Yes, Lck, Abl, Arg, Jak1, Jak2, Jak3, Fak, Pyk2, Fes, Brk, and Syk. In one preferred embodiment, the compounds are used to inhibit the activity of a FGFR, most preferably of FGFR3.

In one preferred embodiment of the invention, the compounds of general formulae (I) and (II), (Ia) and (IIa) are useful for the preparation of a medicament for inhibiting tyrosine kinase associated diseases or disorders including angiogenesis, particularly tumor angiogenesis and tumor progression. In another preferred embodiment of the invention, the compounds are useful for preventing, treating or inhibiting a malignant cell proliferative disease or disorder caused by abnormal PTK activity. According to these two embodiments, the compounds are useful for the treatment or prevention of non-solid cancers, e.g. hematopoietic malignancies such as, but not being limited to, all types of leukemia, e.g. chronic myelogenous leukemia (CML), acute myelogenous leukemia (AML), mast cell leukemia, chronic lymphocytic leukemia and acute lymphocytic leukemia, lymphomas, and multiple myeloma, as well as of solid tumors such as, but not being limited to, mammary, ovarian, prostate, colon, cervical, gastric, esophageal, papillary thyroid, pancreatic, bladder, colorectal, melanoma, small-cell lung and non-small-cell lung cancers, granulosa cell carcinoma, transitional cell carcinoma, vascular tumors, all types of sarcomas, e.g. osteosarcoma, chondrosarcoma, Kaposi's sarcoma, myosarcoma, hemangiosarcoma, and glioblastomas.

It is to be understood that whenever the terms “treating or inhibiting a malignant cell proliferative disease or disorder”, “treating or inhibiting a non-solid cancer”, “treating or inhibiting a tumor” are used herein in the description and in the claims, they are intended to encompass tumor formation, primary tumors, tumor progression or tumor metastasis.

- 5 In another preferred embodiment, the compounds of general formulae (I) and (II), (Ia) and (IIa) of the present invention are used for the preparation of a medicament for the treatment of bone and cartilage related disorders such as a skeletal dysplasia or a craniosynostosis disorder associated with abnormal activation of a receptor protein tyrosine kinase. Examples of craniosynostosis disorders are Muenke coronal craniosynostosis or Crouzon
10 syndrome with acanthosis nigricans. The skeletal dysplasia may be achondroplasia, thanatophoric dysplasia (TD), hypochondroplasia, severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN) dysplasia. In a most preferred embodiment, the compounds are used for treatment of achondroplasia.

- In another embodiment of the present invention, the compounds of general formulae (I)
15 and (II), (Ia) and (IIa) can be used for the preparation of a medicament useful for treating or inhibiting nonmalignant tumors such as benign prostate hypertrophy.

- In a further embodiment, the compounds of general formulae (I) and (II), (Ia) and (IIa) are useful for inhibiting vascular smooth muscle cell proliferation and are thus useful in the treatment of disorders such as atherosclerosis, hypertrophic heart failure and post surgical
20 restenosis.

- In still further embodiments of the present invention, the compounds of general formulae (I) and (II), (Ia) and (IIa) are useful for treatment of autoimmune diseases such as rheumatoid arthritis and inflammatory bowel disease, of vision disorders such as diabetic retinopathy and macular degeneration, of metabolic disorders, of other cell proliferative
25 diseases or disorders such as psoriasis, hypertrophic scars, acne and sclerosis/scleroderma, and of other diseases or disorders such as polyps, multiple exostosis, hereditary exostosis, retrolental fibroplasia, hemangioma, and arteriovenous malformation.

- In one further embodiment of the invention, conjugates of the compounds of general formulae (I) and (II), (Ia) and (IIa) with peptides, antibodies, growth factors or any other
30 entity that can specifically target the compound to the protein kinase of interest are used. The targeting moiety may be linked covalently to the compounds of general formulae (I) and (II), (Ia) and (IIa) either directly or through a linker. For example, if a tyrosine kinase

inhibitor of general formulae (I) and (II), (Ia) and (IIa) is linked to EGF, as described in US 5,911,995, the conjugate will bind to EGFRs present on the surface of a cell and will inhibit tyrosine kinases associated with the EGFR, thereby inducing apoptosis and clonogenic cell death.

- 5 Alternatively, the compounds of the present invention may be delivered conjugated to a polymer (Kratz, et al, 1999).

To determine their biological activity, the compounds of the invention are tested and evaluated in standard assays used to determine inhibition of PTK activity such as described herein in the examples for inhibition of FGFR3 activated by its ligand FGF9 or as

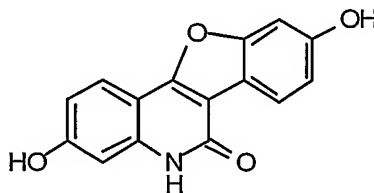
- 10 described, for example, in US 5,733,913, herein incorporated by reference in its entirety as if fully disclosed herein.

The therapeutically effective dose of a compound of the invention to be administered to an individual in need thereof will be sufficient to achieve the desired inhibition and will typically be from about 1 mg to about 100 mg/kg of body weight per day. Typical adult
15 doses will be about 50 to about 800 mg per day, either in single or in multiple doses. The quantity of active component in a unit dose preparation may be varied or adjusted from about 0.1 mg to about 500 mg, preferably about 0.5 mg to 100 mg according to the particular application and the potency of the active component. The composition can, if desired, also contain other compatible therapeutic agents.

- 20 The invention will now be illustrated by the following non-limiting Examples.

EXAMPLES

Example 1. Synthesis of 3,9-dihydroxy-5H-benzofuro[3,2-c] quinoline-6-one (Compound 1)



25 Compound 1

1a) Preparation of 3-(2,4-dimethoxyphenyl)-4-hydroxy-7-methoxy-1H-quinolin-2-one

This intermediate was prepared according to Yamaguchi et al, J. Heterocyclic. Chem., **21**, 737, 1984. A mixture of diethyl 2-(2,4-dimethoxyphenyl)-malonate (1 equiv., 11.53 mmol, 3.4g) and m-methoxyaniline (1 equiv., 12.96 mmol, 1.6g) was refluxed in diphenyl ether (8g) for 2 hours. The cooled reaction mixture was then treated with ether and 5% NaOH aq. The ether layer was extracted with 5 % NaOH aq., and the alkaline layers were combined and acidified with 18% HCl aq to pH 1-2. The white precipitate formed was collected and recrystallized from acetic acid to give the title compound as pale brown needles (m.p. 298-300 °C (lit. m.p. 302-304)). Yield was 0.84 g (25%).

1b) Preparation of Compound 1

10 A mixture of 3-(2,4-dimethoxyphenyl)-4-hydroxy-7-methoxy-1H-quinolin-2-one (2.8 mmol, 0.84 g) obtained in step 1a above and pyridine hydrochloride (7 g) was vigorously refluxed for 2 hours. The reaction mixture was cooled and treated with water (100 ml) to give a white precipitate, which was carefully collected, dried at 70 ° overnight and recrystallized from ethanol to give 0.47 g (62.5%) of Compound 1.

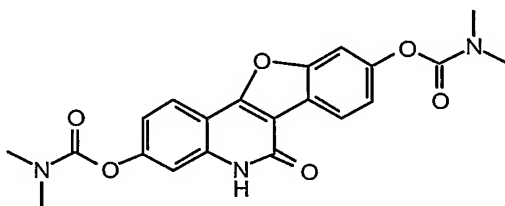
15 m.p. 354°-356°C; (lit. 357°-359°).

NMR (400 MHz): 11.67(s, 1H), 10.24(s, 1H), 9.85(s, 1H), 7.81(d, j=6.8Hz, 1H), 7.79(d, j=6.5Hz, 1H), 7.11 6.89 (m, 2H), 6.79(dd, J1=6.5Hz, J2=1.5Hz, 1H).

MS: molecular peak M⁺ at 268.1 (calculated M+1=268.24)

Example 2. Synthesis of 3,9-bis(N,N-dimethylcarbamoyloxy)-5H-benzofuro[3,2-c]

20 quinoline-6-one (Compound 2)



Compound 2

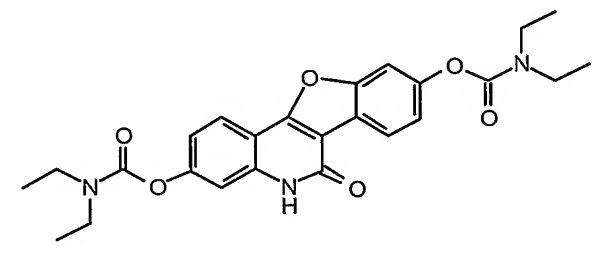
Dimethylcarbamyldichloride (6 equiv., 0.434g) was dissolved in 2 ml DMF and added to the solution of Compound 1 obtained in Example 1 above (1 equiv., 0.90 g), triethylamine (4 equiv., 136 mg) and dimethylaminopyridine (0.1 equiv., 4.1 mg.) in 2 ml DMF. The resulting mixture was stirred at room temperature for 20 hours. After that, the solution was

poured in water (20 ml) and extracted with ether (3 x 25 ml). The ether layer was washed with brine and dried with sodium sulfate. After evaporation of the solvent the product was recrystallized from ethanol to give 0.79 g white powder of Compound 2 (57.6%), m.p. 236-240°C.

- 5 NMR (400 MHz): 12.07(s, 1H), 8.05(d, $j=7.5\text{Hz}$, 1H), 8.04(d, $j=7.2\text{Hz}$, 1H), 7.71(d, $j=1.4\text{Hz}$, 1H), 7.26-6.89 (m, 2H), 7.14(dd, $J_1=7.1\text{Hz}$, $J_2=1.5\text{Hz}$, 1H), 3.10(s, 3H), 3.08(s, 3H), 2.95(s, 6H).

MS: molecular peak M^+ at 410.3 (calculated $M+1=410.4$).

Example 3. Synthesis of Compound 3

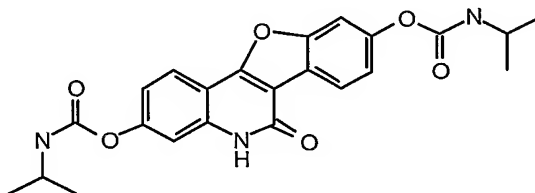


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Compound 3

Compound 3 was prepared by the same procedure as for Compound 2 in Example 2 above, but using diethylcarbamyl chloride instead of dimethylcarbamyl chloride.

- 15 Example 4. Synthesis of Compound 4



Compound 4

Compound 4 was prepared by the same procedure as for Compound 2 in Example 2 above, but using isopropyl isocyanate instead of dimethylcarbamyl chloride and with the

- 20 exclusion of DMAP. See scheme 2 in figure 6.

Example 5. Synthesis of Compound 5Step 1: Synthesis of diethyl 2-(2-methoxy, 4-nitrophenyl) malonate.

2,28 g (94,8 mmol, 2,2 eq) sodium hydride was suspended in 50 mL of dry dioxane, cooled in an ice bath, and 8,28 g (51,72 mmol, 1,2 eq) of diethylmalonate were added slowly.

- 5 After 30 min the suspension had gelled and 20 mL of Dioxane was added and the ice bath removed. After one hour 7,42 g (51,72 mmol, 1,2 eq) of CuBr was added to give a yellow suspension, which turned green after another 30 min. Then 10 g (43,09 mmol) of 2-bromo-5-nitro anisole were added. Afterwards the brown suspension was heated for 10,5 hours at reflux. After cooling down a mixture of 100 mL dil. HCl and ice was carefully added to the
- 10 suspension. Ethyl acetate was added and the phases were separated. The aqueous phase was extracted twice with ethyl acetate, the organic phases were combined and washed with brine and dried over magnesium sulfate. After evaporation of the solvent 14 g of light brown material was left, which upon crystallization from hot hexane gave 9,2 g (29,6 mmol, 67 %) of title compound as yellow crystals. Purity was around 82 % (18 % 2-
- 15 bromo-5-nitro anisole).

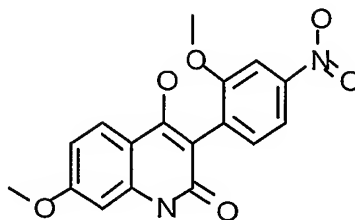
Thin layer chromatography: (ether/ligroin 1/1): R_f : 0,43 Product

$^1\text{H-NMR}$ -Spectrum (500 MHz, CDCl_3): 1,25 (t, 6 H, CH_2CH_3) ppm; 3,97 (s, 3 H, OCH_3) ppm; 4,22 (q, 4 H, CH_2) ppm; 5,19 (s, 1 H, CH) ppm; 7,52 (d, 1 H, Ar-H) ppm; 7,69 (dd, 1 H, Ar-H) ppm; 7,89 (dd, 1 H, Ar-H) ppm.

- 20 $^{13}\text{C-NMR}$ -Spectrum (125 MHz, CDCl_3): 13,9 (CH_2CH_3) ppm; 51,06 (CH) ppm; 56,23 (OCH_3) ppm; 62,07 (CH_2) ppm; 105,54 (Ar-CH) ppm; 115,76 (Ar-CH) ppm; 128,93 (Ar-C) ppm; 130,18 (Ar-CH) ppm; 148,61 (Ar-C) ppm; 157,30 (Ar-C) ppm; 167,40 (Carbonyl) ppm

Step 2: Synthesis of 3-(2-methoxy, 4-nitro phenyl)-4-hydroxy-7-methoxy-1H-quinolin-2-

- 25 one (compound 5)

**Compound 5**

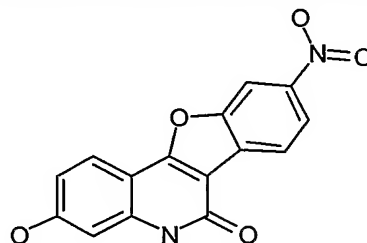
The title compound was synthesized by mixing up 5 g (13,17 mmol of 82 % pure material) of diethyl 2-(2-methoxy, 4-nitrophenyl) malonate together with 1,62 g (1 eq) of m-anisidine and 8 g of diphenylether. The mixture was heated for three hours under reflux with an air condenser. After cooling down the mixture was dissolved in 100 mL of ether and extracted 5 four times with 50 mL of a 5 % sodium hydroxide solution. The aqueous phases were combined and cooled in an ice bath. Around 50 mL of an 18 % hydrochloric acid were added to bring the solution to a pH of 1 – 2. The white material, which precipitated from the solution was separated, dried and re-crystallized from 220 mL of acetic acid to give 2,1 g (6,1 mmol, 46 %) of the title compound.

10 ^1H -NMR-Spectrum (500 MHz, DMSO- D^6): 3,90 (t, 6 H, OCH_3) ppm; 6,79 (d, 1 H, Ar-H) ppm; 6,82 (s, 1 H, Ar-H) ppm; 7,40 (d, 1 H, Ar-H) ppm; 7,78 (s, 1 H, Ar-H) ppm; 7,82 (dd, 1 H, Ar-H) ppm; 7,89 (dd, 1 H, Ar-H) ppm; 10,20 (s, 1 H, NH) ppm; 11,39 (s, 1 H, OH) ppm

^{13}C -NMR-Spectrum (125 MHz, DMSO- D^6): 55,36 (OCH_3) ppm; 55,99 (OCH_3) ppm ;
 15 97,82 (Ar- $\underline{\text{CH}}$) ppm; 115,76 (Ar- $\underline{\text{CH}}$) ppm; 105,24 (Ar-C) ppm; 105,72 (Ar- $\underline{\text{CH}}$) ppm; 108,94 (Ar-C) ppm; 109,94 (Ar- $\underline{\text{CH}}$) ppm; 114,96 (Ar- $\underline{\text{CH}}$) ppm; 124,97 (Ar- $\underline{\text{CH}}$) ppm; 130,53 (Ar-C) ppm; 140,23 (Ar-C) ppm; 147,88 (Ar-C) ppm; 158,45 (Ar- $\underline{\text{C}}$ - OCH_3) ppm; 158,52 (Ar- $\underline{\text{C}}$ - OCH_3) ppm; 161,42 ($\underline{\text{C}}$ -OH) ppm; 162,29 (Carbonyl) ppm

Step 3: Synthesis of 3-hydroxy 9-nitro-5H-benzofuro[3,2-c] quinoline-6-one (compound 6)

20



Compound 6

3-(2-methoxy, 4-nitro phenyl)-4-hydroxy-7-methoxy-1H-quinolin-2-one (2,1 g, 6,14
 25 mmol) was mixed with 9,93 g (85,9 mmol, 7 eq for each methoxy-group) of freshly sublimed pyridinium hydrochloride. The mixture was heated for 2,5 hours at 200° C with an air condenser. After cooling down, the mixture was treated with 100 mL of water. The dark green suspension was filtered, the solid was washed with water and dried to give 1,7 g of a dark green material. This material was filtrated with ethanol on a short silica gel (8
 30 cm, 60 mesh) column. The fractions with the same spot in the TLC were combined and

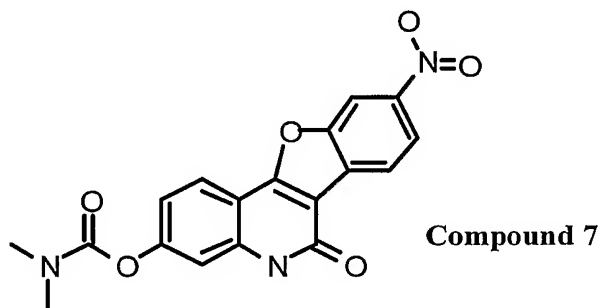
evaporated to dryness to leave 490 mg (1,65 mmol; 27 %) of a red-orange material having a melting point over 320° C.

Thin layer chromatography (Ethanol): R_f : 0,62 (red-yellow) Product

Mass Spectra (EI): M^+ : 296; 100 %

5 Step 4: Synthesis of 3- methylcarbamoyloxy 9-nitro-5H-benzofuro[3,2-c] quinoline-6-one
– (Compound 7)

10

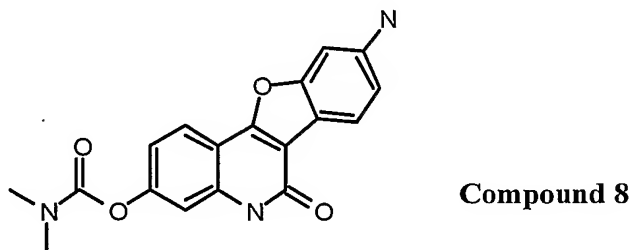


Compound 7 was synthesized by dissolving 490 mg (1,65 mmol) of the material from step 3 (3-hydroxy 9-nitro-5H-benzofuro[3,2-c] quinoline-6-one; compound 6) in 1,5 mL of dry DMF. With stirring at room temperature, 0,67 g (4 eq) of triethylamine, and 20,2 mg (0,1
 15 eq) of 4-N,N-dimethylaminopyridine were added. Then a solution of 1,07 g (9,9 mmol, 6 eq) of dimethylcarbamoyl chloride in 1,5 mL of dry DMF was added dropwise over 10 min. The solution was stirred for 29 hours at room temperature. Afterwards the red suspension was carefully diluted with 10 mL of water and a light brown precipitate separated, which was washed successive with water, ether and dried to leave 376 mg (1,02
 20 mmol; 62 %) of light brown material.

Mass Spectra (EI): M^+ : 367.

Step 5: Synthesis of 3-methylcarbamoyloxy 9-amino-5H-benzofuro[3,2-c] quinoline-6-one
(Compound 8)

25



This synthesis was adapted from Boothroyd, S. R. and Kerr, M. A Tetrahedron Letters 36 (1995) 2411.

360 mg (0,98 mmol) of compound 4 were suspended in 2 mL of methanol, 25 mg of charcoal, 3,5 mg (10,51 mmol) of Iron-III-Chloride hexahydrate and 0,8 mL (10,51 mmol) of N,N-dimethylhydrazine were added while stirring. The suspension was heated for 22 hours under reflux. Afterwards most of the solvent was removed under vacuum. The brown viscous residue was flash chromatographed on silica gel (60 mesh) with methanol/dichloromethane 1/10 (vol.). The liquid phase was changed after fraction 14 to methanol/dichloromethane 1/1 (vol.). Yield was 31 mg of the title compound.

Mass Spectra (EI): M^+ : 337.

Example 6. Biological activity tests

10 **6a) FDCP cell line and transfections**

The FDCP cell line (ATCC no. CRL-12103) is a murine immortalized IL-3-dependent cell line of myelocytic bone marrow origin, which does not express endogenous FGF receptors (FGFRs). FDC-P1 (FDCP) cells were cultured at 37 °C in a humidified atmosphere containing 5% CO₂. The cells were grown in Iscove's modified Dulbeccos's medium (IMDM) supplemented with 10% fetal calf serum (FCS), 2mM L-glutamine, 100U/ml penicillin, and 100µg/ml streptomycin, and with 1% X-63-0 supernatant containing IL-3 (or with 0.1 ng/ml IL-3 from Pepro Tech).

FDCP cells were transfected with either the human wild-type FGF receptor 3 (FGFR3; FDCP-FR3), or with the human wild-type FGF receptor 1 (FGFR1; FDCP-FR1). PAW-FGFR1 construct (from Dr. M. Mohammadi, see Mohammadi et al., 1997) was used for stable transfection of FDCP cells. Twenty million (20×10^6) cells were used for each transfection. The cells were washed and resuspended in 800 µl of IMDM without FCS. 50 µg DNA was added to the cells prior to electroporation (315v, 97 µF). The cells were cooled on ice for 10 minutes and resuspended in IMDM-FCS medium supplemented with 1% X-63-0 supernatant. 48 hours later medium was exchanged and 1mg/ml G418 (GibcoBRL) was added.

Retroviral Infection: FGFR3 was constructed in a viral expression system using pLXSN (Clontech). FDCP cells were infected by MuLV containing pLXSN-FGFR constructs. 0.5×10^6 FDCP cells were incubated for 3 hours with 0.5 ml viral supernatant (and polybrene 4 µg/ml). The infected cells were cultured with IMDM-FCS medium containing IL-3. G418 (1 mg/ml) was added 24h post infection.

6b) Stimulation of cells by growth factors

FDCP transfected /infected cells were grown in medium containing IL-3 washed with medium without IL-3 prior to FGF stimulation. Stimulation of the cells by growth factors was performed by addition of 10 ng/ml FGF9 and 5 µg/ml heparin to the medium (without
5 IL-3). Cells were grown with FGF+ heparin with media replaced every two days.

6c) FDCP Cell Proliferation Assay

Upon transfection with a FGFR cDNA, FGF induces a dose-dependent proliferation of the FDCP cell line, and this proliferative effect of FGF can replace the IL-3 growth dependence of the FDCP cells. FDCP cell lines transfected with FGFR cDNA can,
10 therefore, be used for screening of specific inhibitors or activators of FGFs, or for studying mutant FGFRs and FGFR signaling. The effect of various ligands on FDCP cell proliferation is determined by a cell proliferation assay using XTT reagent according to the manufacturer's instructions (Cell Proliferation Kit, Biological Industries Co., Beit Haemek, Israel). The method is indicative of cell viability, and it is based on the capability of
15 mitochondrial enzymes to reduce tetrazolium salts into soluble colored formazan compounds, which can be then quantitated.

FDCP cells were grown in Iscove's medium containing 2 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 12.5 µg/ml nystatin, 10% FBS (Iscove's+++), 10 ng/ml FGF9, and 5µg/ml heparin. The medium containing heparin and FGF was prepared
20 every 7-10 days. Under these culture conditions, the cells exhibited a "pearl-like" appearance, i.e., they were round and shiny.

A day prior to the experiment, the cells were split 1:3 with fresh Iscove's+++ medium containing 5µg/ml heparin and 10 ng/ml FGF9. Twenty four hours later the cells were washed twice in Iscove's+++ medium, and resuspended at a final concentration of 4×10^5
25 cells/ml in Iscove's+++ medium containing 5µg/ml heparin. Fifty µl of cell suspension/well were seeded in a 96-well plate (2×10^4 cells/well).

The tested compounds were usually prepared at a concentration of 10 mM in 100% DMSO (stock solution), from which serial dilutions were made in DDW (a final concentration of no more than 0.125% DMSO per well was added). At the final dilution the compounds
30 were prepared in Iscove's+++ medium containing 5 µg/ml heparin and 2.5 ng/ml FGF9 or IL-3. Fifty µl were added to each well and the plate was incubated at 37°C. After 48 hrs of incubation, 50 µl of the XTT mixture was added to each well. The absorbance (O.D.) at

490 nm at this time point gave the baseline reading. Cells were then incubated for additional 4 hours at 37⁰C, and the proliferation was measured by absorbance at 490 nm. It should be noted that addition of DMSO alone did not affect cell proliferation.

The compounds are further tested to determine the level of inhibition and specificity of inhibition by performing in vivo assays to a variety of cell types expressing different receptor tyrosine kinase proteins and by in vitro kinase assays known in the art.

“Receptor specificity” as referred to herein denotes the fact that a certain compound inhibits a biological response (e.g. proliferation) elicited by a particular receptor at an IC₅₀ that is at least half of that required to inhibit the response elicited by other receptors.

Biological responses and receptor affinity are measured by methods known in the art.

Results FDCP Proliferation Assay with Compounds 2, 5, 6, 7, and 8.

The receptor tyrosine kinase inhibitory activity of Compound 2 was assayed using the FDCP proliferation assay described in Example 5b above. Increasing concentrations of Compound 2 were added either to FDCP-FR3 cells or to FDCP-FR1 cells that were grown in the presence of either FGF9 (F9) or IL-3. Two days later, an XTT proliferation assay was performed. The results are shown in Fig. 1 and demonstrate that Compound 2 inhibits FGF9-dependent proliferation of FDCP-FR3 (R3) cells or FDCP-FR1 (R1) cells mediated by FGFR3 or FGFR1, respectively. Moreover, the IL-3-dependent cell proliferation was not affected by this inhibitor, demonstrating that Compound 2 is not toxic at the range of concentrations used here, and that it is specific for the FGF receptors. The table in figure 5 presents the IC₅₀ of compounds (compounds 5-8) synthesized according to Example 5 above. The compounds were tested in the in vivo FDCP cell assay for inhibition of FGFR3 activity in FGFR3 transfected FDCP cells. The IC₅₀ was determined by calculating the concentration of compound required to inhibit 50% proliferation. Compound 6, 3-hydroxy 9-nitro-5H-benzofuro[3,2-c) quinoline-6-one, and compound 8, 3-methylcarbamoxyloxy 9-amino-5H-benzofuro[3,2-c) quinoline-6-one, show the highest level of protein tyrosine kinase inhibition, while compound 7 showed the least.

6d) FGFR3 Expression and Activation in RCJ Cells

RCJ cells (fetal rat calvaria-derived mesenchymal cells, RCJ 3.1C5.18; Grigoriadis *et al.*, 1988) were generated to express various FGF Receptors an inducible manner, in the absence of tetracycline, as described (Monsonogo-Oman *et al.*, 2000). The cells were incubated in medium containing a low serum concentration and FGF was added to

stimulate receptor activity and signaling. The cells were lysed and the receptor (FGFR3) level, the receptor activation and signaling were assessed by Western blotting using anti-FGFR3, anti-phosphotyrosine, or anti-active JUN kinase (JNK) antibodies, respectively.

RCJ-W11 cells (RCJ cells stably transfected to overexpress wild type FGFR3) were
5 grown to subconfluence in α -MEM containing 2 mM glutamine, 100 U/ml penicillin, 100 μ g/ml streptomycin, 12.5 μ g/ml nystatin (medium A) and supplemented with 600 μ g/ml neomycin, 2 μ g/ml tetracycline, 50 μ g/ml hygromycin B and 15% fetal calf serum (FCS). The cells were trypsinized and seeded (6×10^5 cells/well in a 6-well plate) in the
aforementioned medium, excluding the hygromycin B. Twenty four hours later the cells
10 were washed and then incubated with medium A supplemented with 15% FCS for 16 hours. Cells were washed and allowed to grow for 4 additional hours in 1 ml of medium A containing 0.5% serum.

The test compounds were usually prepared at a concentration of 10 mM in 100% DMSO (stock solution), from which serial dilutions were made in DDW (a final concentration of
15 no more than 0.125% DMSO per well was added).

RCJ-W11 cells were incubated in the presence of the test compounds for 20 min prior to FGF9 stimulation (5 min at a concentration of 100 ng/ml). Thereafter, the cells were placed on ice, washed twice with ice-cold PBS, and then lysed with lysis buffer (0.5 ml; EGTA 1 mM, EDTA 1 mM, Tris 25 mM, Hepes 50 mM, NaF 25 mM, β -glycerophosphate 50 mM,
20 NaCl 50 mM, glycerol 10%, NP40 1%, pH 7.5, protease inhibitor mixture). The lysate was then microcentrifuged (10 minutes at 4⁰C), and the cleared lysate was collected. The protein concentration was measured and the samples were prepared for 7.5% SDS-PAGE using Laemmli sample buffer. Alternatively, 10 μ l anti-FGFR3 antibodies (Anti-FGFR3, SantaCruz #123) were added to the lysate (0.5 ml) and incubated for 4 hours at 4⁰C. Forty
25 μ l protein A-Sepharose™ were added and incubated for an additional 1 hour at 4⁰C with continuous shaking. The mixture was then microcentrifuged (15 seconds), and the supernatant was aspirated. The beads were washed with lysis buffer and the samples were prepared for 7.5% SDS-PAGE using Laemmli sample buffer.

Following gel electrophoresis, the proteins were transferred onto nitrocellulose paper using
30 the Mini trans-blot electrophoretic transfer cell (BioRad #170-3930, #170-3935). Anti-FGFR3 or Anti-JNK were To detect the proteins the ECL detection kit was used. (PIERCE, Supersignal-WestPico #34080).

Results Inhibition of FGFR3 activation

The receptor tyrosine kinase inhibitory activity of Compound 2 was assayed by testing its effect on FGF9 stimulation of FGFR3 phosphorylation and its downstream signal transduction activation of JNK as described above in Example 6d. Fig. 2 shows a Western blot of the immunoprecipitated FGFR3 probed with antibodies against either FGFR3 (R3) or against phosphotyrosine antibodies (pR3). In addition, cell lysates of the above treated cells were analyzed by Western blot using anti-P-JNK antibodies (JNK). The results in Fig. 2 demonstrate that Compound 2 inhibits both the FGF9-induced FGFR3 tyrosine phosphorylation and the FGF9-induced JNK activation in RCJ-W11 cells.

10 6e) *Ex vivo* Bone Culture

Femoral bone cultures were prepared by excising the hind limbs of heterozygous mice for the achondroplasia G369C mutation (age P0) (Gly369Cys mutation in mouse FGFR3 which causes achondroplasia, equivalent to the G380C mutation in humans; see Chen *et al.*, 1999). The limbs were carefully cleaned from the surrounding tissue (skin and muscles), the femora removed, and further cleared from tissue remains and ligaments. The femora were measured for their initial length using a binocular equipped with an eyepiece micrometer ruler. The bones were grown in 1 ml of medium (α -MEM supplemented with 100 U/ml penicillin, 0.1 mg/ml streptomycin, 12.5 units/ml nystatin, 0.2% BSA, 1 mM β -glycerophosphate, and 50 μ g/ml freshly prepared ascorbic acid) in a 24-well tissue culture plate. A stock solution of the tested compound was usually prepared at a concentration of 10 mM in 100% DMSO. The final dilution of the compound was made directly into the well such that a final concentration of no more than 0.125% DMSO per well was added. The bones were cultured for 9 days. Measurements of bone length, medium replacement, and compound replacement were performed every three days. At the end of the experiment, the growth rate of the bones was calculated based on the length measurements obtained during day 3 to day 9.

Results: Effect of Compound 2 on bone growth in organ culture.

The results presented in Fig. 3 demonstrate that the addition of Compound 2 accelerates the growth of Ach369 femora as compared with the untreated femora. In addition, comparison of the growth rate during day 3 to day 9 reveals that Compound 2 increases the growth rate of the femoral bone by about 2 folds as compared to the untreated femora (4.33 vs. 2 units/day respectively) (Fig.4).

Example 7: Synthetic Schemes

The following schemes provide the synthetic pathway for certain preferred compounds and scaffolds.

Scheme 1 (figure 6) discloses the pathway for synthesizing compounds of structural
5 formula I.

Scheme 2 (figure 7) presents the pathway for the synthesis of compound II and derivatives having structural formula II.

While the present invention has been particularly described, persons skilled in the art will appreciate that many variations and modifications can be made. Therefore, the invention is
10 not to be construed as restricted to the particularly described embodiments, rather the scope, spirit and concept of the invention will be more readily understood by reference to the claims which follow.

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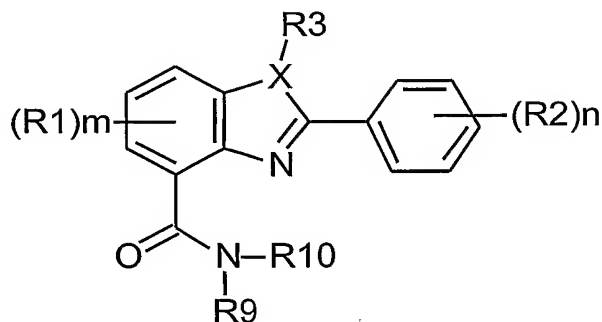
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CLAIMS:

1) A protein kinase inhibitor having the general formula (I):

**I**

or a stereoisomer or a pharmaceutically acceptable salt thereof; wherein

5 X is N or O;

R1 and R2 are at each occurrence independently selected from halogen, nitro, cyano, trifluoromethyl, hydrocarbyl, OR4, SR4, SOR5, SO₂R5, COOH, COR6, SONR7R8, SO₂NR7R8 and NR7R8;

R3 is selected from H or R1, and is absent when X is O;

10 R9 and R10 are independently selected from H and R1;

R4 is selected from H, hydrocarbyl, COR6, and CONR7R8;

R5 is hydrocarbyl;

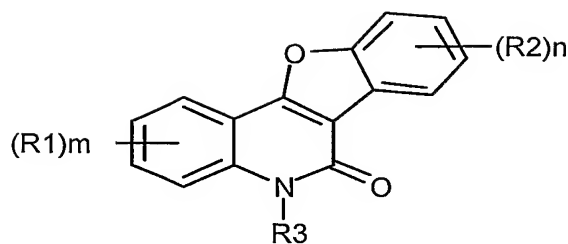
R6 is selected from H, hydrocarbyl, OR5 and NR7R8;

15 R7 and R8 are each independently selected from H or hydrocarbyl, or one of R7 or R8 is H or hydrocarbyl and the other is COR5, COOR5 or CONR7R8, or R7 and R8 together with the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and sulfur;

m is 0 to 3 and n is 0 to 5;

20 with the proviso that a) when m is 0, at least one of R3, R9 or R10 is other than H; and b) when X is O, at least one of R9 or R10 is other than H.

2) A protein kinase inhibitor having the general formula (II):



II

or a stereoisomer or a pharmaceutically acceptable salt thereof; wherein

R1 and R2 are at each occurrence independently selected from halogen, nitro, cyano, trifluoromethyl, hydrocarbyl, OR4, SR4, SOR5, SO₂R5, COOH, COR6, SONR7R8, SO₂NR7R8 and NR7R8;

R3 is H or R1;

R4 is selected from H, hydrocarbyl, COR6, and CONR7R8;

R5 is hydrocarbyl;

R6 is selected from H, hydrocarbyl, OR5 and NR7R8;

R7 and R8 are each independently selected from H or hydrocarbyl, or one of R7 or R8 is H or hydrocarbyl and the other is COR5, COOR5 or CONR7R8, or R7 and R8 together with the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and sulfur;

m and n independently are an integer from 0 to 4;

with the proviso that when R3 is H and m is 0, 1 or 2 and n is 0 or 1, R1 is other than C₁-C₁₀ alkyloxy, COOH, CONH₂, CO(CH₂)₂₋₇OH, OR4, N-mono-alkylcarbamoyl, N,N-di-alkylcarbamoyl, alkylsulfonyl or CHO.

3) The protein kinase inhibitor according to claim 1 wherein X is N, m is 1, R1 at position 5 is a radical NHCOCH₃, R9 and R10 are H, R3 is CH₂-CH₂-COOH, CH₂-CH₂-COOR5, or CH₂-CH₂-CONR7R8, wherein R5 is C₁-C₈ alkyl, preferably methyl, and R7 and R8 are each independently selected from H or hydrocarbyl or R7 and R8 together with the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected

from oxygen, nitrogen and sulfur, n is 2 and R₂ is C₁-C₈ alkoxy, preferably methoxy, most preferably at positions 3 and 5 of the phenyl radical.

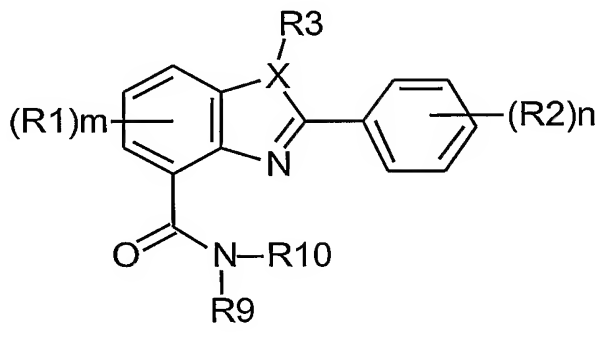
4) The protein kinase inhibitor according to claim 3 having the structure 3-(5-acetylamino-4-carbamoyl-2-(3,5-dimethoxyphenyl)-benzimidazol-1-yl)-propionic acid.

5) The protein kinase inhibitor according to claim 2 wherein R₃ is H, m is 1, R₁ is selected from OH or dimethyl carboxamoyl, n is 2, R₂ is selected from NO₂ or NH₂.

10

6) The protein kinase inhibitor according to claim 5 selected from the group consisting of (a) 3-hydroxy 9-nitro-5H-benzofuro[3,2-c] quinoline-6-one; (b) 3-methylcarbamoyloxy 9-amino-5H-benzofuro[3,2-c] quinoline-6-one.

15 7) A pharmaceutical composition comprising as an active ingredient a protein kinase inhibitor having the general formula (I):



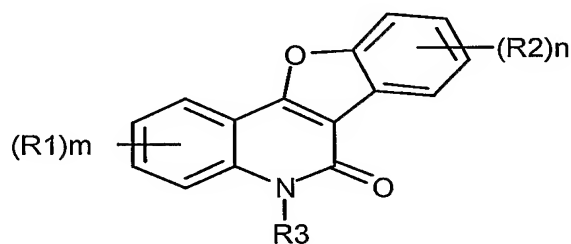
or a stereoisomer or a pharmaceutically acceptable salt thereof; wherein

X is N or O;

20 R₁ and R₂ are at each occurrence independently selected from halogen, nitro, cyano, trifluoromethyl, hydrocarbyl, OR₄, SR₄, SOR₅, SO₂R₅, COOH, COR₆, SONR₇R₈, SO₂NR₇R₈ and NR₇R₈;

R₃ is selected from H or R₁, and is absent when X is O;

- R9 and R10 are independently selected from H and R1;
 R4 is selected from H, hydrocarbyl, COR6, and CONR7R8;
 R5 is hydrocarbyl;
 R6 is selected from H, hydrocarbyl, OR5 and NR7R8;
- 5 R7 and R8 are each independently selected from H or hydrocarbyl, or one of R7 or R8 is H or hydrocarbyl and the other is COR5, COOR5 or CONR7R8, or R7 and R8 together with the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and sulfur;
- 10 m is 0 to 3 and n is 0 to 5;
- with the proviso that a) when m is 0, at least one of R3, R9 or R10 is other than H; and
 b) when X is O, at least one of R9 or R10 is other than H;
- and a pharmaceutically acceptable carrier, diluent or excipient.
- 15 8) A pharmaceutical composition comprising as an active ingredient a protein kinase inhibitor having the general formula (II):

**II**

- or a stereoisomer or a pharmaceutically acceptable salt thereof; wherein
- R1 and R2 are at each occurrence independently selected from halogen, nitro, cyano,
 20 trifluoromethyl, hydrocarbyl, OR4, SR4, SOR5, SO₂R5, COOH, COR6, SONR7R8, SO₂NR7R8 and NR7R8;
- R3 is H or R1;
- R4 is selected from H, hydrocarbyl, COR6, and CONR7R8;
- R5 is hydrocarbyl;

R6 is selected from H, hydrocarbyl, OR5 and NR7R8;

R7 and R8 are each independently selected from H or hydrocarbyl, or one of R7 or R8 is H or hydrocarbyl and the other is COR5, COOR5 or CONR7R8, or R7 and R8 together with the nitrogen atom to which they are attached form a saturated or

5 unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and sulfur;

m and n independently are an integer from 0 to 4;

with the proviso that when R3 is H and m is 0, 1 or 2 and n is 0 or 1, R1 is other than C₁-C₁₀ alkyloxy, COOH, CONH₂, CO(CH₂)₂₋₇OH, OR4, N-mono-alkylcarbamoyl, N,N-

10 di-alkylcarbamoyl, alkylsulfonyl or CHO;

and a pharmaceutically acceptable carrier, diluent or excipient.

9) The pharmaceutical composition according to claim 7 wherein X is N, m is 1, R1 at position 5 is a radical NHCOCH₃, R9 and R10 are H, R3 is CH₂-CH₂-COOH, CH₂-CH₂-COOR5, or CH₂-CH₂-CONR7R8, wherein R5 is C₁-C₈ alkyl, preferably methyl,

15 and R7 and R8 are each independently selected from H or hydrocarbyl or R7 and R8 together with the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and sulfur, n is 2 and R2 is C₁-C₈ alkoxy, preferably methoxy,

20 most preferably at positions 3 and 5 of the phenyl radical.

10) The pharmaceutical composition according to claim 9 comprising as an active ingredient 3-(5-acetylamino-4-carbamoyl-2-(3,5-dimethoxyphenyl)-benzimidazol-1-yl)-propionic acid.

25

11) The pharmaceutical composition according to claim 8 comprising as an active ingredient a compound wherein R3 is H, m is 1, R1 is selected from OH or dimethyl carboxamoyl, n is 2, R2 is selected from NO₂ or NH₂.

12) The pharmaceutical composition according to claim 11 comprising as an active ingredient a compound selected from the group consisting of (a) 3-hydroxy 9-nitro-5H-benzofuro[3,2-c] quinoline-6-one; (b) 3-methylcarbamoyloxy 9-amino-5H-benzofuro[3,2-c] quinoline-6-one.

5

13) The pharmaceutical composition according to claims 7 or 8 for inhibition of a cell proliferative or metabolic disease or disorder caused by abnormal PTK activity.

14) The pharmaceutical composition according to claim 13 for inhibition of angiogenesis, and tumor angiogenesis.

10

15) The pharmaceutical composition according to claim 13 for treating or inhibiting a nonmalignant or malignant cell proliferative disease or disorder caused by abnormal PTK activity.

16) The pharmaceutical composition according to claim 15 for the prevention, treatment or inhibition of non-solid cancers or solid tumors.

15

17) The pharmaceutical composition according to claim 15 for preventing, treating or inhibiting tumor formation, primary tumors, tumor progression or tumor metastasis.

20

18) The pharmaceutical composition according to claim 13 for treatment of bone and cartilage related disorders.

19) The pharmaceutical composition according to claim 18 for treatment of a skeletal dysplasia or a craniosynostosis disorder associated with abnormal activation of a RPTK.

25

20) The pharmaceutical composition according to claim 19 wherein said craniosynostosis disorder is Muenke coronal craniosynostosis or Crouzon syndrome with acanthosis nigricans.

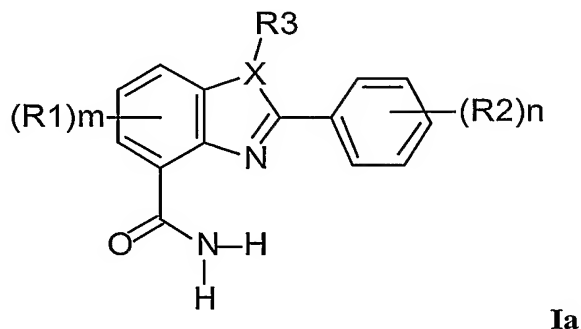
21) The pharmaceutical composition according to claim 19 wherein the skeletal dysplasia is selected from achondroplasia, thanatophoric dysplasia (TD), hypochondroplasia, severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN) dysplasia.

5

22) The pharmaceutical composition according to claim 21, wherein the skeletal dysplasia is achondroplasia.

23) The pharmaceutical composition according to claims 7 or 8 comprising a conjugate of a compound of the structural formula I or II with an entity selected from a peptide, an antibody, a growth factor or any other entity that can specifically target the compound to a protein kinase of interest, wherein said targeting moiety may be linked covalently to the compound either directly or through a linker.

24) Use of a compound of general formula (Ia):



wherein

X is N or O;

R1 and R2 at each occurrence are independently selected from halogen, nitro, cyano, trifluoromethyl, hydrocarbyl, OR4, COOH, COR6 and NR7R8;

R3 is H or R1, and is absent in Formula I at position 1 when X is O;

R4 is selected from H, hydrocarbyl, COR6, and CONR7R8;

R5 is hydrocarbyl;

R6 is selected from H, hydrocarbyl, OR5 and NR7R8;

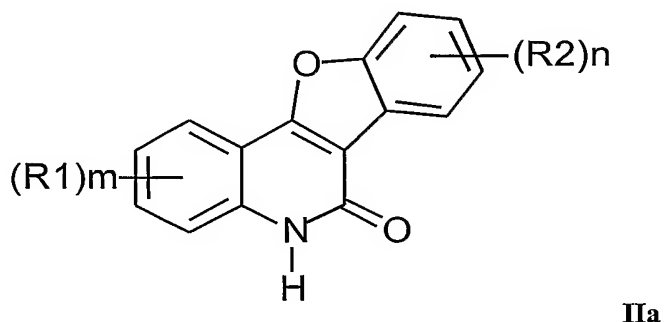
R7 and R8 are each independently selected from H or hydrocarbyl, or one of R7 or R8 is H or hydrocarbyl and the other is COR5, COOR5 or CONR7R8, or R7 and R8 together with the nitrogen atom to which they are attached form a saturated or
 5 unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and sulfur; and

m is 0 to 3 and n is 0 to 5;

for the preparation of a medicament for treating or preventing protein tyrosine kinase related diseases and disorders.

10

25) Use of a compound of general formula (IIa):



wherein

15 R1 and R2 are each independently selected from halogen, hydrocarbyl, OR4, SR4, SOR5, SO₂R5, COOH, COR6, SONR7R8, SO₂NR7R8 and NR7R8;

R4 is selected from H, hydrocarbyl, COR6, and CONR7R8;

R5 is hydrocarbyl;

R6 is selected from H, hydrocarbyl, OR5 and NR7R8;

20 R7 and R8 are each independently selected from H or hydrocarbyl, or one of R7 or R8 is H or hydrocarbyl and the other is COR5, COOR5 or CONR7R8, or R7 and R8 together with the nitrogen atom to which they are attached form a saturated or

unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and sulfur; and
m and n are independently selected from an integer of 0 to 4;
for the preparation of a medicament for treating or preventing protein tyrosine kinase
5 related diseases and disorders.

26) Use according to claim 24 wherein m is 1 and X is N or O.

27) Use according to claim 25, wherein R3 is hydrogen.

10

28) Use according to claim 27, wherein R1 and R2 are OR4, wherein R4 is hydrogen or
OCONR7R8 wherein R7 and R8 are each C₁-C₃ alkyl, and each of m and n is 1.

29) Use according to claim 27, wherein m and n are 1 and R1 and R2 are OH.

30) Use according to claim 27, wherein R1 and R2 are both OCON(CH₃)₂.

15 31) Use according to claims 24 or 25 for inhibition of a cell proliferative, skeletal or
metabolic disease or disorder caused by abnormal PTK activity.

32) Use according to claim 30 for inhibition of angiogenesis, particularly tumor
angiogenesis.

20 33) Use according to claim 30 for treating or inhibiting a nonmalignant or malignant
cell proliferative disease or disorder caused by abnormal PTK activity.

34) Use according to claim 32 for the prevention, treatment or inhibition of non-solid
cancers or solid tumors.

35) Use according to claim 32 for preventing, treating or inhibiting tumor formation,
primary tumors, tumor progression or tumor metastasis.

25 36) Use according to claim 32 for treatment of bone and cartilage related disorders.

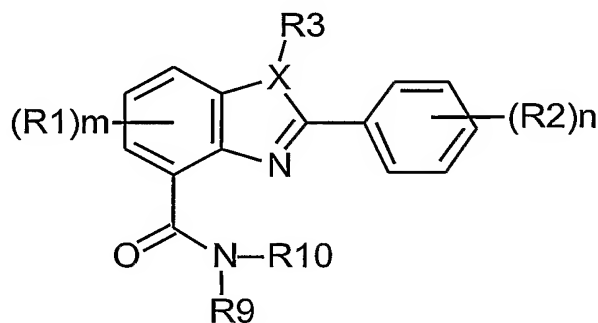
37) Use according to claim 35 for treatment of a skeletal dysplasia or a
craniosynostosis disorder associated with abnormal activation of a RPTK.

38) Use according to claim 36 wherein said craniosynostosis disorder is Muenke coronal craniosynostosis or Crouzon syndrome with acanthosis nigricans.

39) Use according to claim 36 wherein the skeletal dysplasia is selected from achondroplasia, thanatophoric dysplasia (TD), hypochondroplasia, severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN) dysplasia.

40) Use according to claim 38, wherein the skeletal dysplasia is achondroplasia.

41) A method for treating a protein tyrosine related disease or disorder which comprises administering to a subject in need of such treatment a therapeutically effective amount of a pharmaceutical composition having protein tyrosine kinase inhibitory activity which comprises as an active ingredient a therapeutically effective quantity of a compound of general formula (I):



or a stereoisomer or a pharmaceutically acceptable salt thereof; wherein

15 X is N or O;

R1 and R2 are at each occurrence independently selected from halogen, nitro, cyano, trifluoromethyl, hydrocarbyl, OR4, SR4, SOR5, SO₂R5, COOH, COR6, SONR7R8, SO₂NR7R8 and NR7R8;

R3 is selected from H or R1, and is absent when X is O;

20 R9 and R10 are independently selected from H and R1;

R4 is selected from H, hydrocarbyl, COR6, and CONR7R8;

R5 is hydrocarbyl;

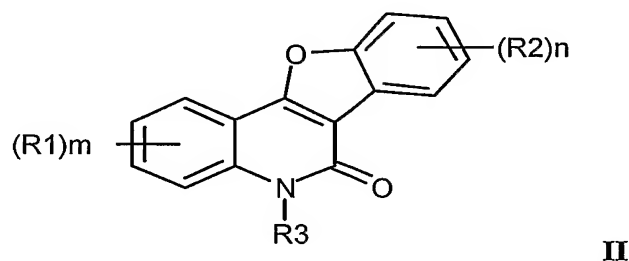
R6 is selected from H, hydrocarbyl, OR5 and NR7R8;

R7 and R8 are each independently selected from H or hydrocarbyl, or one of R7 or R8 is H or hydrocarbyl and the other is COR5, COOR5 or CONR7R8, or R7 and R8 together with the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and sulfur;

m is 0 to 3 and n is 0 to 5;

with the proviso that a) when m is 0, at least one of R3, R9 or R10 is other than H; and b) when X is O, at least one of R9 or R10 is other than H.

42) A method for treating a protein tyrosine related disease or disorder which comprises administering to a subject in need of such treatment a therapeutically effective amount of a pharmaceutical composition having protein tyrosine kinase inhibitory activity which comprises as an active ingredient a therapeutically effective quantity of a compound of general formula (II):



or a stereoisomer or a pharmaceutically acceptable salt thereof; wherein

R1 and R2 are at each occurrence independently selected from halogen, nitro, cyano, trifluoromethyl, hydrocarbyl, OR4, SR4, SOR5, SO₂R5, COOH, COR6, SONR7R8, SO₂NR7R8 and NR7R8;

R3 is H or R1;

R4 is selected from H, hydrocarbyl, COR6, and CONR7R8;

R5 is hydrocarbyl;

R6 is selected from H, hydrocarbyl, OR5 and NR7R8;

R7 and R8 are each independently selected from H or hydrocarbyl, or one of R7 or R8 is H or hydrocarbyl and the other is COR5, COOR5 or CONR7R8, or R7 and R8 together with the nitrogen atom to which they are attached form a saturated or

unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and sulfur;

m and n independently are an integer from 0 to 4;

with the proviso that when R₃ is H and m is 0, 1 or 2 and n is 0 or 1, R₁ is other than
5 C₁-C₁₀ alkyloxy, COOH, CONH₂, CO(CH₂)₂₋₇OH, OR₄, N-mono-alkylcarbamoyl, N,N-di-alkylcarbamoyl, alkylsulfonyl or CHO.

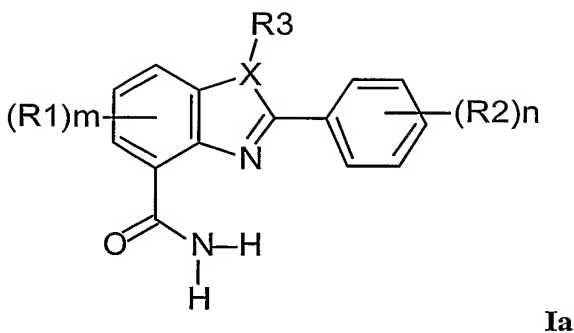
43) The method according to claim 41 wherein the pharmaceutical composition comprises as active ingredient a compound wherein X is N, m is 1, R₁ at position 5 is a radical NHCOCH₃, R₉ and R₁₀ are H, R₃ is CH₂-CH₂-COOH, CH₂-CH₂-COOR₅, or
10 CH₂-CH₂-CONR₇R₈, wherein R₅ is C₁-C₈ alkyl, preferably methyl, and R₇ and R₈ are each independently selected from H or hydrocarbyl or R₇ and R₈ together with the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and sulfur, n is 2 and R₂ is C₁-C₈ alkoxy, preferably methoxy, most preferably at positions 3
15 and 5 of the phenyl radical.

44) The method according to claim 43 wherein the pharmaceutical composition comprises as active ingredient 3-(5-acetylamino-4-carbamoyl-2-(3,5-dimethoxyphenyl)-benzimidazol-1yl)-propionic acid.

45) The method according to claim 42 wherein the pharmaceutical composition
20 comprises as active ingredient a compound wherein R₃ is H, m is 1, R₁ is selected from OH or dimethyl carboxamoyl, n is 2, R₂ is selected from NO₂ or NH₂.

46) The method according to claim 45 wherein the pharmaceutical composition comprises as active ingredient a compound selected from the group consisting of (a) 3-hydroxy 9-nitro-5H-benzofuro[3,2-c] quinoline-6-one; (b) 3-methylcarbamoyloxy 9-
25 amino-5H-benzofuro[3,2-c] quinoline-6-one.

47) A method for treating a protein tyrosine related disease or disorder which comprises administering to a subject in need of such treatment a therapeutically effective amount of a pharmaceutical composition having protein tyrosine kinase inhibitory activity which comprises as an active ingredient a therapeutically effective
30 quantity of a compound of general formula (Ia):



wherein

X is N or O;

R1 and R2 at each occurrence are independently selected from halogen, nitro, cyano,
5 trifluoromethyl, hydrocarbyl, OR4, COOH, COR6 and NR7R8;

R3 is H or R1, and is absent in Formula I at position 1 when X is O;

R4 is selected from H, hydrocarbyl, COR6, and CONR7R8;

R5 is hydrocarbyl;

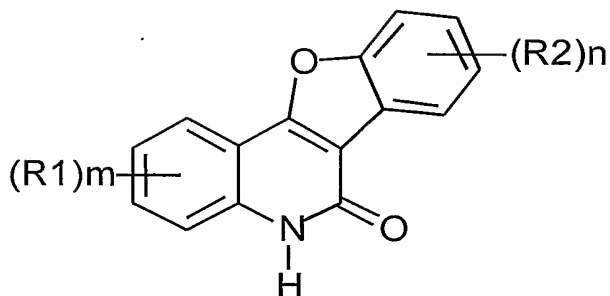
R6 is selected from H, hydrocarbyl, OR5 and NR7R8;

10 R7 and R8 are each independently selected from H or hydrocarbyl, or one of R7 or R8
is H or hydrocarbyl and the other is COR5, COOR5 or CONR7R8, or R7 and R8
together with the nitrogen atom to which they are attached form a saturated or
unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected
from oxygen, nitrogen and sulfur; and

15 m is 0 to 3 and n is 0 to 5;

for the preparation of a medicament for treating or preventing protein tyrosine kinase
related diseases and disorders.

48) A method for treating a protein tyrosine related disease or disorder which
comprises administering to a subject in need of such treatment a therapeutically
20 effective amount of a pharmaceutical composition having protein tyrosine kinase
inhibitory activity which comprises as an active ingredient a therapeutically effective
quantity of a compound of general formula (IIa):



IIa

wherein

R1 and R2 are each independently selected from halogen, hydrocarbyl, OR4, SR4, SOR5, SO₂R5, COOH, COR6, SONR7R8, SO₂NR7R8 and NR7R8;

5 R4 is selected from H, hydrocarbyl, COR6, and CONR7R8;

R5 is hydrocarbyl;

R6 is selected from H, hydrocarbyl, OR5 and NR7R8;

R7 and R8 are each independently selected from H or hydrocarbyl, or one of R7 or R8 is H or hydrocarbyl and the other is COR5, COOR5 or CONR7R8, or R7 and R8

10 together with the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from oxygen, nitrogen and sulfur; and

m and n are independently selected from an integer of 0 to 4;

for the preparation of a medicament for treating or preventing protein tyrosine kinase
15 related diseases and disorders.

49) The method according to claim 47 wherein m is 1 and X is N or O.

50) The method to claim 48, wherein R3 is hydrogen.

51) The method to claim 50, wherein R1 and R2 are OR4, wherein R4 is hydrogen or OCONR7R8 wherein R7 and R8 are each C₁-C₃ alkyl, and each of m and n is 1.

20 52) The method according to claim 50, wherein m and n are 1 and R1 and R2 are OH.

53) The method according to claim 50, wherein R1 and R2 are both OCON(CH₃)₂.

54) The method according anyone of claims 41-53 for inhibition of a cell proliferative or skeletal disease or disorder caused by abnormal PTK activity.

- 55) The method according to claim 54 for inhibition of angiogenesis, particularly tumor angiogenesis.
- 56) The method according to claim 54 for treating or inhibiting a nonmalignant or malignant cell proliferative disease or disorder caused by abnormal PTK activity.
- 5 57) The method according to claim 54 for the prevention, treatment or inhibition of non-solid cancers or solid tumors.
- 58) The method according to claim 57 for preventing, treating or inhibiting tumor formation, primary tumors, tumor progression or tumor metastasis.
- 59) The method according to claim 54 for treatment of bone and cartilage related
10 disorders.
- 60) The method according to claim 59 for treatment of a skeletal dysplasia or a craniosynostosis disorder associated with abnormal activation of a RPTK.
- 61) The method according to claim 60 wherein said craniosynostosis disorder is Muenke coronal craniosynostosis or Crouzon syndrome with acanthosis nigricans.
- 15 62) The method according to claim 60 wherein the skeletal dysplasia is selected from achondroplasia, thanatophoric dysplasia (TD), hypochondroplasia, severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN) dysplasia.
- 63) The method according to claim 62, wherein the skeletal dysplasia is
20 achondroplasia.
- 64) The method according to claim 54, wherein said PTK is a receptor protein tyrosine kinase (RPTK) selected from the group consisting of FGFR1, FGFR2, FGFR3, FGFR4, FGFR5, EGFR/ErbB1, ErbB2/HER2/Neu, ErbB/HER3, ErbB4/HER4, IGF-1R, PDGFR- α , PDGFR- β , CSF-1R, kit/SCFR, Flk2/FH3, Flk/VEGFR1, Flk1/VEGFR2,
25 Flt4/VEGFR3, TrkA, TrkC, HGFR, RON, EphA2, EphB2, EphB4, Axl, TIE/TIE1, Tek/TIE2, Ret, ROS, and Alk, or a cytoplasmic PTK selected from the group consisting of Src, Fgr, Yes, Lck, Abl, Arg, Jak1, Jak2, Jak3, Fak, Pyk2, Fes, Brk, and Syk.
- 65) The method according to claim 64 for inhibition of a cell proliferative, skeletal or metabolic disease or disorder caused by abnormal PTK activity.

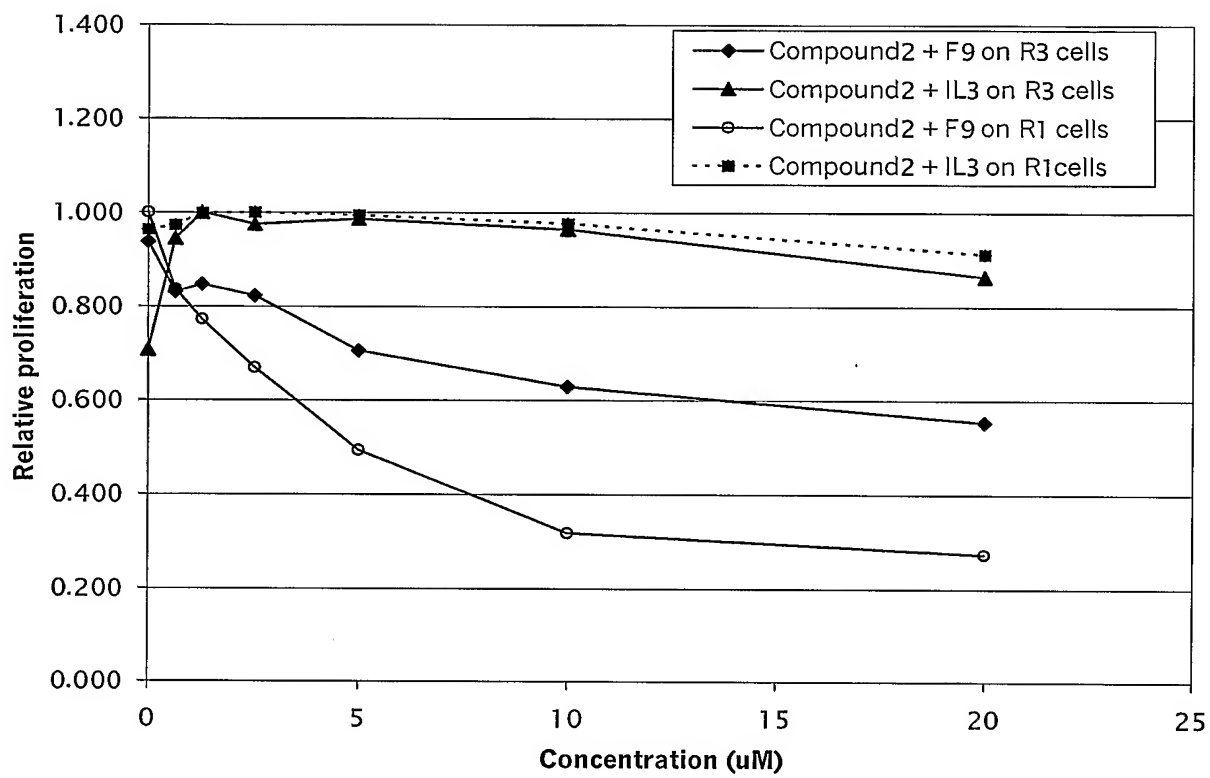
Figure 1

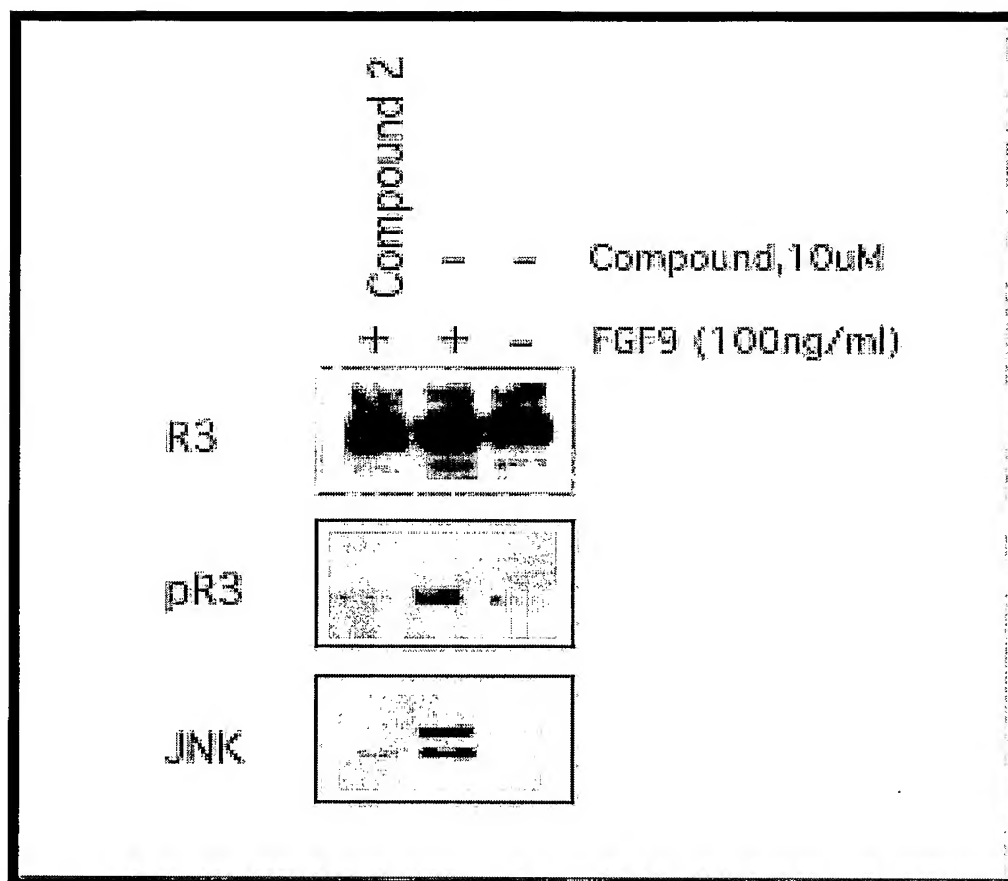
Figure 2

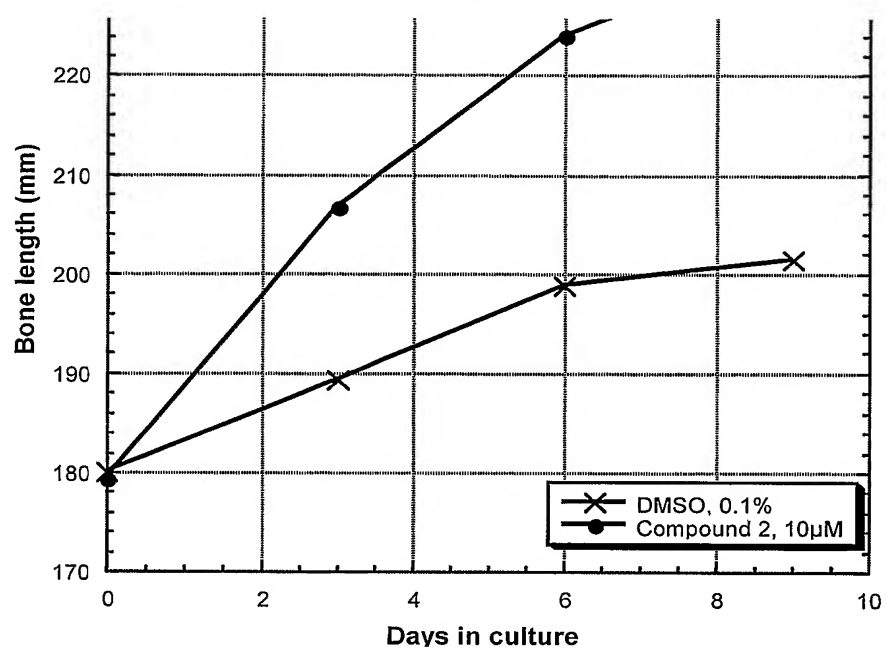
Figure 3

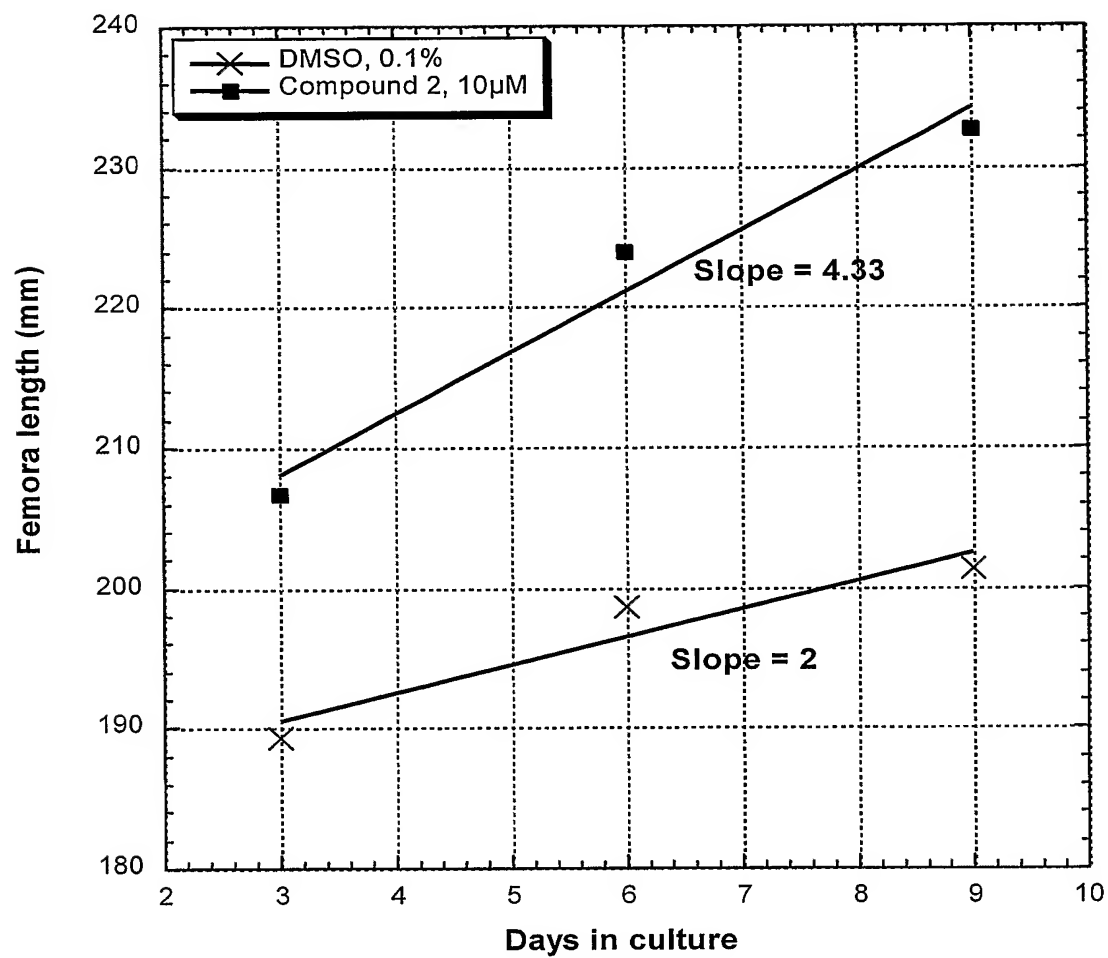
Figure 4

Figure 5

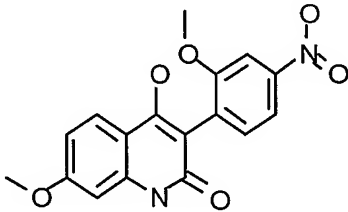
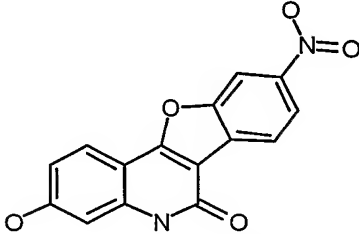
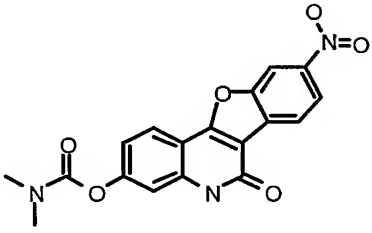
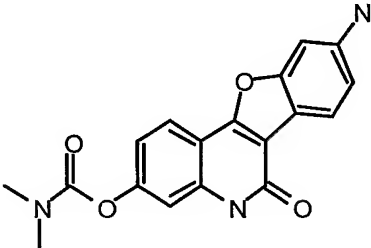
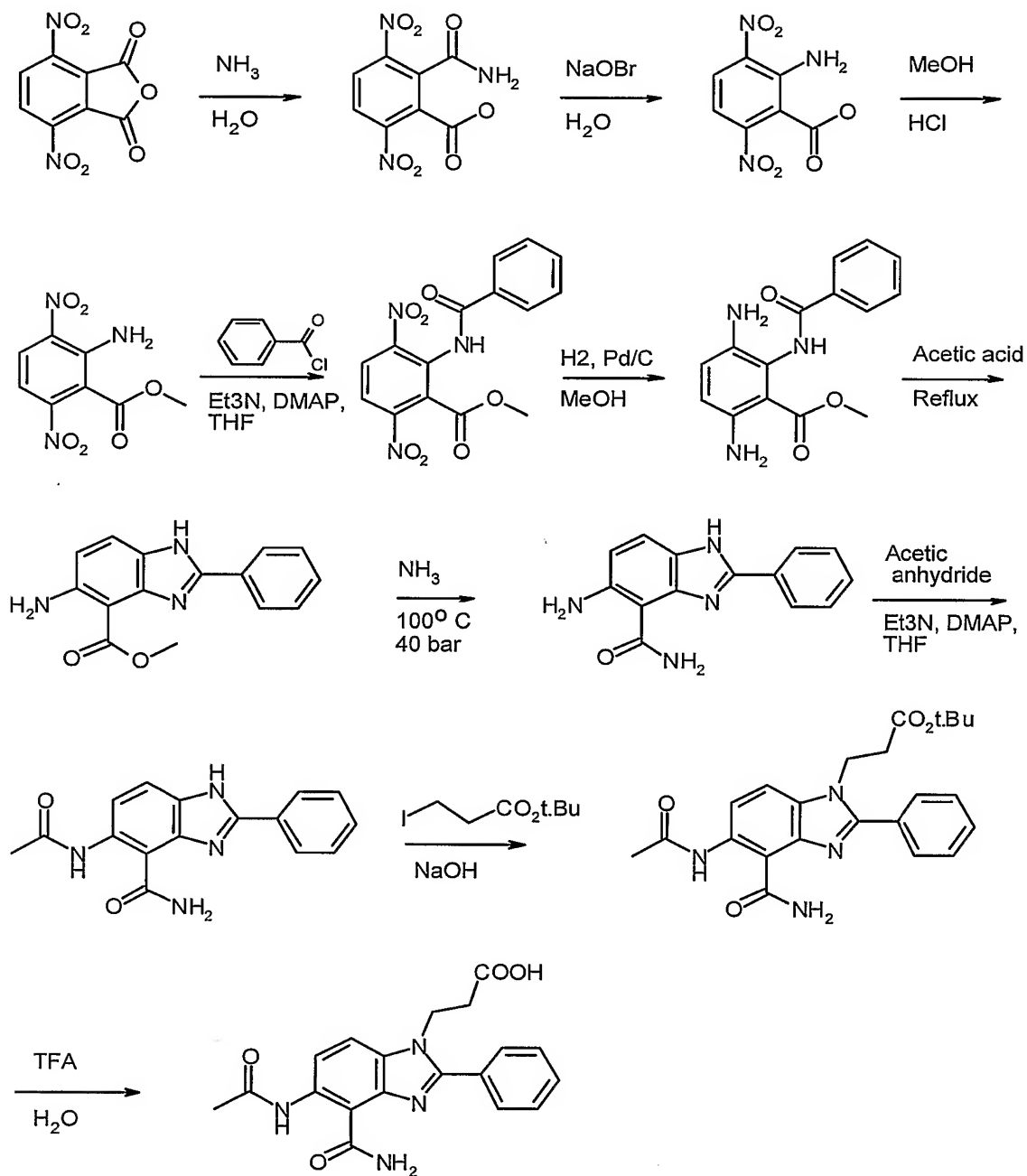
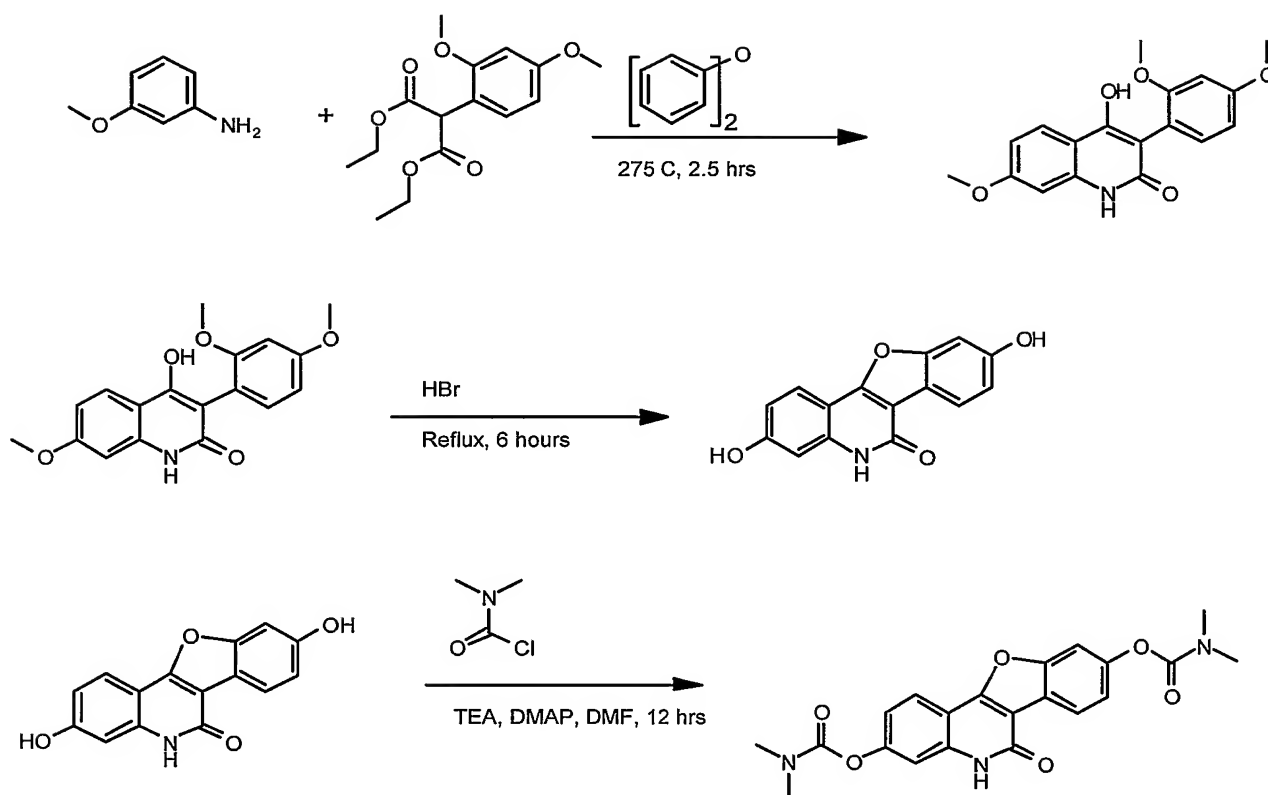
<u>Compound #</u>	<u>Structure</u>	<u>formula</u>	<u>IC50</u>
5		C ₁₇ H ₁₆ N ₂ O ₆	15 uM
6		C ₁₅ H ₁₀ N ₂ O ₅	7 uM
7		C ₁₈ H ₁₅ N ₃ O ₆	<20 uM
8		C ₁₈ H ₁₅ N ₃ O ₄	10 um

Figure 6



Scheme 1

Figure 7**Scheme 2**

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
13 March 2003 (13.03.2003)

PCT

(10) International Publication Number
WO 2003/020698 A3

- (51) International Patent Classification⁷: **A61K 31/4184**, C07D 235/18 (74) Agent: WEBB, Cynthia; Webb & Associates, P.O. Box 2189, 76121 Rehovot (IL).
- (21) International Application Number: PCT/IL2002/000740 (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
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- (71) Applicant (*for all designated States except US*): **PROCHON BIOTECH LTD.** [IL/IL]; P.O. Box 1482, 76114 Rehovot (IL).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **EREN, Doron** [IL/IL]; Moshav Netaim, 46870 (IL). **ZALIANI, Andrea** [DE/DE]; Dorotheenstrasse 23, 22301 Hamburg (DE). **PE'ER, David** [IL/IL]; Hanasi Harishon Street 34, Rehovot 76303 (IL). **BOGIN, Oren** [IL/IL]; Moshav Ganei Yochanan 160, 76922 (IL). **YAYON, Avner** [IL/IL]; Moshav Sitria # 104, 76834 (IL).
- Published:
— with international search report
- (88) Date of publication of the international search report: 18 March 2004
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: PROTEIN TYROSINE KINASE INHIBITORS

(57) Abstract: The present invention provides heterocyclic compounds which can act as protein tyrosine kinase inhibitors. Preferred embodiments include both novel and known substituted 2-aryl benzimidazole and benzofuro [3,2-c] quinoline compounds. Methods of utilizing preferred compounds in preparation of medicaments for the treatment of proliferative, skeletal and metabolic diseases and disorders associated with abnormal protein tyrosine kinase activity, including cancer and skeletal dysplasia, are provided.



WO 2003/020698 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL02/00740

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/4184; C07D 235/18

US CL : 514/394; 548/310.7

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/394; 548/310.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/26192 A1 (BASF AKTIENGESELLSCHAFT) 11 May 2000 (11/05/00), see entire document, specially compound 17 on page 40.	1, 7, 13-24, 24, 26, 31, 41, 47, 49, 54-60, 64, 65
X	WO 97/04771 A1 (NEWCASTLE UNIVERSITY VENTURES LIMITED) 13 February 1997 (13/02/1997), see entire documents, especially compound NU1090 on page 46.	1, 7, 13-24, 26, 31, 41, 47, 49, 54-58, 64, 65
X	DENNY et al. Potential Antitumor Agents. 59. Structure-Activity Relationships for 2-Phenylbenzimidazole-4-carboxamides, a New Class of "Minimal" DNA-Intercalating Agents Which May Not Act via Topoisomerase II. J. Med. Chem., February 1990, Vol. 33, No. 2, pages 814-819, see especially the compounds in Table II on page 816.	1, 7, 13-24, 26, 31, 41, 47, 49, 54-58, 64, 65



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:		"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier application or patent published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

07 April 2003 (07.04.2003)

Date of mailing of the international search report

20 MAY 2003

Name and mailing address of the ISA/US

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Authorized officer

Laura L. Stockton, Ph.D.

Telephone No. 703/308-1235

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL02/00740

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1, 3, 4, 7, 9, 10, 13-24, 26, 31, 41, 43, 44, 47, 49 and 54-65 (in part)

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

PCT/IL02/00740

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

Group I, claim(s) 1, 3, 4, 7, 9, 10, 13-24, 26, 31, 41, 43, 44, 47, 49 and 54-65, drawn to products of formula (I) wherein X is N and methods of use.

Group II, claim(s) 1, 7, 13-24, 26, 31, 41, 47, 49 and 54-64, drawn to products of formula (I) wherein X is O and methods of use.

Group III, claim(s) 2, 5, 6, 8, 11-23, 25, 27-40, 42, 45, 46, 48 and 50-65, drawn to products of formula (II) and methods of use.

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: There is a lack of unity among the above identified groups because there is no significant structural element shared by all of the alternatives. Each of the groups set forth above represents a separate discrete heterocyclic ring system which one skilled in the art which beside sharing no significant structural element, cannot be said to belong to a recognized class of chemical compounds in the pharmaceutical art. The claims are therefore considered to lack unity of invention.